MINISTRY OF EDUCATION AND TRAINING
NONG LAM UNIVERSITY
FACULTY OF FOOD SCIENCE AND TECHNOLOGY

INTERNATIONAL WORKSHOP ON
FOOD SAFETY AND PROCESSING TECHNOLOGY

Ho Chi Minh City, November 29-30, 2007
CONFERENCE AGENDA

November 29th, 2007

08:00 – 08:30: Welcome and Registration

08:30 – 08:45: Opening speech

08:45 – 09:15: Key speaker

09:15 – 10:05: Series of concurrent sessions

10:05 – 10:25: Tea break

10:25 – 11:40: Series of concurrent sessions (cont’)

12:00 – 13:30: Lunch time

13:30 – 14:45: Series of concurrent sessions (cont’)

14:45 – 15:00: Tea break

15:00 – 16:15: Series of concurrent sessions (cont’)

November 30th, 2007

8:00 – 17:00: Free tour (optional)

Ho Chi Minh City:

Option 1: Ben Thanh Market, China Town,

Museum of War Memory

Option 2: Cu Chi War Tunnel
Series of concurrent sessions

Session 1: Food Safety, Properties and Applications
Chairmans: Dr. Luu Duan, Dr. Athapol Noomhorm

<table>
<thead>
<tr>
<th>Time</th>
<th>Paper</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:15-09:40</td>
<td>Preservation of Grape (<em>Vitis vinifera</em> L.) by Postharvest Calcium Treatment</td>
<td>T. Anh Pham</td>
</tr>
<tr>
<td>09:40-10:05</td>
<td>Study On Technological Processing of Instant Corn Gruel</td>
<td>M. Hung Le</td>
</tr>
<tr>
<td>10:05-10:25</td>
<td>Tea break</td>
<td></td>
</tr>
<tr>
<td>10:25-10:50</td>
<td>A Modified Automatic Hydroponics System As Model S. Tuan Nguyen Technology For Producing Safe-Vegetables</td>
<td></td>
</tr>
<tr>
<td>10:50-11:15</td>
<td>Study On Quality of Thai Aromatic Rices</td>
<td>A. Noomhorm</td>
</tr>
<tr>
<td>11:15-11:40</td>
<td>Survival of Fortified <em>Escherichia coli</em>, <em>Staphylococcus aureus</em>, T. Long Truong and <em>Listeria monocytogenes</em> in <em>Nem Chua</em> Produced by Traditional Technology and Modified Technology</td>
<td></td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch time</td>
<td></td>
</tr>
<tr>
<td>13:30-13:55</td>
<td>Situation of Abattoirs for Bovines and Pigs in SE-Asia and Recommendations for Urgently Needed Improvements</td>
<td>Gunter Heinz</td>
</tr>
<tr>
<td>13:55-14:20</td>
<td>Application of MPN - PCR (Most Probable Number - Polymerase Chain Reaction) to enumerate <em>Clostridium botulinum</em> spores in honey</td>
<td>T. L. An Vu</td>
</tr>
<tr>
<td>14:20-14:45</td>
<td>Aloe (<em>Aloe Vera</em> L.) Fermentation Process</td>
<td>T. M. Nguyen</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>Tea break</td>
<td></td>
</tr>
<tr>
<td>15:00-15:25</td>
<td>Study On Determining Harvesting Indexes of Some Specific Fruits In Order To Improve Their Quality In Preservation and Processing</td>
<td>V. H. Ha Nguyen</td>
</tr>
<tr>
<td>15:50-16:15</td>
<td>Study On Appropriate Packing and Optimum Storage Temperature For Some Specific Fruits</td>
<td>V. H. Ha Nguyen, M. Hung Le</td>
</tr>
</tbody>
</table>
Concurrent of sessions

Session 2: Novel Food Processing and Preservation Technology
Chairmans: Truong Thanh Long, Dr. Reynes Max

<table>
<thead>
<tr>
<th>Time</th>
<th>Paper</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:15-09:40</td>
<td>Advances in High Pressure Processing of Foods</td>
<td>Roman Buckow</td>
</tr>
<tr>
<td>09:40-10:05</td>
<td>Effect of Mild-Heat and High-Pressure Processing on Banana Pectin Methylesterase: A Kinetic Study</td>
<td>N. Binh Ly</td>
</tr>
<tr>
<td>10:05-10:25</td>
<td>Tea break</td>
<td></td>
</tr>
<tr>
<td>10:25-10:50</td>
<td>Ultrasound Assisted Hydration of Paddy Rice</td>
<td>P. J. Torley</td>
</tr>
<tr>
<td>10:50-11:15</td>
<td>Emergency Technologies</td>
<td>Reynes Max</td>
</tr>
<tr>
<td>11:15-11:40</td>
<td>Near Infrared Evaluation Of Total Soluble Solids And Dry Matter Content In Dragon Fruit (Hylocereus Undatus Haw.)</td>
<td>H.Y.Phuong Pham</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch time</td>
<td></td>
</tr>
<tr>
<td>13:30-13:55</td>
<td>Kinetic Modeling Of 5-Methyl And 5-Formyltetrahydrofolic Acid Degradation During Temperature And High Pressure Treatments</td>
<td>M. T. Nguyen</td>
</tr>
<tr>
<td>13:55-14:20</td>
<td>Functional Properties of Edible Bilayer Films Composed of Polysacharides and Shellac for Food Quality Preservation</td>
<td>T. Dong Phan</td>
</tr>
<tr>
<td>14:20-14:45</td>
<td>Design And Development Laboratory Scale Microwave Vacuum Evaporator Of Juice Concentrate</td>
<td>A. Noomhorne</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>Tea break</td>
<td></td>
</tr>
<tr>
<td>15:00-15:25</td>
<td>Good Agricultural Practices for fresh fruit and vegetables in Vietnam</td>
<td>Q. Vong Nguyen</td>
</tr>
<tr>
<td>15:25-15:50</td>
<td>Food Structure and Novel Processing Tools</td>
<td>Volker Heinz</td>
</tr>
<tr>
<td>15:50-16:15</td>
<td>Microencapsulation of Lactic Acid Bacteria Isolated from Commercial Yogurts In Taiwan</td>
<td>H. Quang Luong</td>
</tr>
</tbody>
</table>
Session 1
Food Safety, Properties and Applications
PRESERVATION OF GRAPE (*Vitis vinifera L.*) BY POSTHARVEST CALCIUM TREATMENT

Pham Tuan Anh, Le Thi Bich
Faculty of Food Technology, Nong Lam University Ho Chi Minh City

ABSTRACT

The postharvest calcium treatment was applied in the recent years to prolong the shelf-life of fruit. In this method, the fruit is dipped into a solution of calcium salt for a time. In this research the influence of two factors, the concentration of calcium chloride in dipping solution and the dipping time, on grape variety NH-01-48 was investigated. Each factor was examined at 4 levels, 0%, 5%, 1%, 1.5%, 2% for concentration and 30, 90, 150, 210 seconds for dipping time. During the experiment, weight, total soluble solid, firmness, vitamin C, and 3 sensory properties: color, appearance and flavor were determined. The result shows that these two factors greatly influence the quality of the grape during preservation. The treated samples always obtain better quality in comparison with the controlled one. The effect of concentration of CaCl2 is more important than dipping time. Among the treatments, dipping the grape in solution of 1% of CaCl2 in 210 seconds gives the best result.

INTRODUCTION

In the recent years, thanks to the promotive policy of the government, the application of scientific methods and the effort of farmers, the production of grape in Ninh Thuan increases quickly, and this fruit contributes considerably to the economical development of the region. With high yield, good quality, high commercial value, the variety of NH-01-48, an adaptive one of imported variety of grape, is the first choice of the farmer in this province (Research Center of Cotton, 1998).

The grape contains a lot of water, nutrients, and specially, sugars. So it is a good media for the microorganisms (Kanelliss et al., 1993). This is why grape is considered as perishable fruit although it is a non-climateric one (Wills, 1981). If we don’t apply appropriate measure, the quality of grape degrade easily and decrease quickly.

Till now, Ninh Thuan doesn’t have yet effective methods and equipments to preserve this commodity. All the production must be consumed at harvest period. If the commercialization isn’t well done, a common event in Viet Nam, a lot of grape is in danger, the farmers have to suffer postharvest loss. This influences poorly to the revenues of the farmer, and in some extend, to the sustainable development of the grape.

For conservation of fruit and vegetable in developed countries, refrigeration, modified atmosphere, . . . are applied with good result. But these methods require high investment, demand skillful operator, high cost. So these methods are inadequate for most vietnamese farmers.

A rather new method has been recently studied and developed for preservation of fruit is calcium treatment. This is a simple method, the fruit is dipped in a solution containing Ca 2+ for a time, ion calcium penetrates into the fruit and some positive changes in the structure and metabolism take place (Thompson, 1996, Stanley, 1991, Yuen, 1994). These effects prolong the ripening process and increase the shelf life of fruit.
The objective of this research is investigating the effect of two factors, concentration of calcium chloride in dipping solution and dipping time, on the preservation process and some properties of grape. From the result obtained, the most appropriate dipping regime can be determined.

**MATERIALS AND METHODS**

In this investigation, grape of NH-01-48 variety was used. It is a very popular one in Ninh Thuan. In each treatment about 500 g of grape with high homogeneity of dimension, color and ripeness was used. The grape is well rinsed, drained, then dipped in CaCl2 solution. The concentration of CaCl2 and dipping time depend on the treatment.

After dipping, the grape is preserved in cool room Dagard TechnoBloc. The temperature and humidity of the cool room are maintained consecutively at 10°C and 90% in 20 days. Each 4 days, samples of treatments are taken to determine physical, biochemical parameters, and to evaluate sensorial changes (in comparison with initial state).

The experiment consisted of 2 stages. The preliminary one is used to determine the appropriate intervals of 2 factors. The result of this stage is used to plan the second one. In the second stage, the full factorial arrangement was applied, 4 levels of CaCl2 concentration were combined with 4 levels of dipping time, so 16 treatments of grape are realized. For comparison purpose, a control treatment (without dipping) is added.

The observed physical and biochemical parameters are:

+ Weight ratio $W_d/W_o$: the ratio between the sample weight at day $d$ $W_d$ and the initial weight $W_o$. These values are determined by Kern scale with precision of 0.01 g.
+ Total soluble solid (TSS): determined by desiccation method following standard TCVN 3700-90 with oven Memmert 500.
+ Firmness: measured by Penetrometer with conic head and maximum force is 5 kG.
+ Vitamin C content: determined by 2-6 diclorophenol indophe nol method following standard TCVN 6427-2-1998.

Sensorial comparison between samples of day $d$ and that of initial state was carried out following standard TCVN 3215–79. The panel composed of 7 panelists. Color, appearance and flavor are observed properties.

The calculation and treatment of raw data is realized with the help of MS Excel 2000. Statistical analysis is carried out with SPSS ver. 7.5.1. A 3D graph is realized with Minitab Release 12.

**RESULTS AND DISCUSSION**

The result of the preliminary stage shows that the most appropriate intervals for concentration of calcium chloride is 0.5% - 2%, and for dipping time is 30 – 200 seconds. So the principal experiment is carried out with 16 treatments by the combination of 4 levels of concentration of CaCl2 : 0.5%, 1%, 1.5% and 2% with 4 levels of dipping time : 30 s, 90 s, 150 s, 210 s (and a controltreatment in addition).

**Change of physical and biochemical parameters**

After the experiment, the data obtained in all 17 treatments are treated and analyzed. But in order to simplify and clarify this section and the next one, these simplifications and conventions are applied:
For each parameter, only the result of 4 treatments are presented in the figures. They are the best (BT), the worst (WT), the average (AT) and the control (CT) treatments. The best treatment is presented with continuous thick line, the worst with continuous thin line, the average with the thin dot line, the control with the thick dash-dot line. In the parentheses following a treatment is the concentration of calcium chloride of dipping solution and the dipping time corresponding to this treatment; for example: AT (1.5%, 150 s).

The difference between treatments exists only in the case that this difference is statistically significant with the observed significance level smaller than 0.05.

**Weight**

In the preservation, the weight of fruit is decreased due to the evaporation, the metabolism (respiration). We can observe this decrease in all treatments, but in different rates. After 20 days, the BT (1%, 210s) decrease only 4% meanwhile this decrease was nearly 7% in the WT (0.5%, 210s). This value for CT is about 10% (Wd/Wo in Fig. 1).

**Total soluble solid**

During postharvest preservation, a considerable quantity of soluble solid in fruit is consumed by physiological and biochemical process, respiration for example. We can recognized this phenomenon in experiment clearly. From initial value of about 16%, after 20 days in cool room, it becomes 14.5% in the BT (1%, 210s), 12.5% in WT (0.5%, 90s) and only 10.5% in CT (TSS in Fig. 1).

**Firmness**

Because protopectin is changed to pectin, the firmness of grape is decreased gradually in preservation (Kays, 1991). The effect of calcium expresses clearly in this property, all the treated samples preserved the firmness better in comparison with control one (Fig. 1).

**Vitamin C content**

We can realize easily that the general trend is that the quantity of Vitamin C reduces in preserving time due to oxygenation (Kays, 1991). The highest losses at day 20 are observed in CT with 2.5 mg % and WT (0.5%, 150s) with 1.8 mg%. It seems that BT (1%, 210s) is an exception with only 0.2 mg% (Fig. 1).

**Change of sensorial properties during preservation**

Similarly to recently discussed parameters, the physiological and biochemical processes provoke also the sensorial changes in the grape during preservation, and we can recognize these changes through Fig. 2.

From Fig. 2, we can realized that color, appearance and flavor of grape change during the preservation; but with different rates. All the treated samples have slower rates in comparison with the control one. Among 16 treatments, the best one in all three properties is the same, the treatment with 1% of CaCl2 in solution and dipping time 210s.
Fig. 1. Changes of physical and biochemical parameters during preservation of grape.

Fig. 2. Changes in sensorial properties during preservation of grape.
Discussion about effect of treatment
From the experiment, we can recognize that the samples treated with calcium always obtain better physical, biochemical and sensorial properties. This result can be explained that in dipping process in calcium solution, Ca\(^{2+}\) penetrates into the grape and the properties of cells and intercellular spaces change. An important reaction is the combination of ion calcium with pectin to form pectate calcium. So spatial nets are created, the structure of the membranes and enclosing components of fruits becomes more rigid, the material exchange through the cells and the fruits are hindered. The rates of principal physiology and biochemical processes are reduced remarkably. For example, the intensity of respiration is reduced by the deficiency of oxygen and the redundant of CO\(_2\), so the consumption of materials is reduced. On the other hand, the evaporation is more difficult, the transpiration lessen, the weight loss reduce, the appearance of fruit can be maintained for a longer time, . . .(Thompson, 1996). Thanks to these effects, the ripening process is slow down, the shelf life of food is prolonged considerably.

Effect of factors on changes
A synthesis of ANOVA of the changes of properties discussed above is shown in Table 1.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of CaCl(_2)^{(a)}</td>
</tr>
<tr>
<td>Weight</td>
<td>0,000</td>
</tr>
<tr>
<td>Total soluble solid</td>
<td>0,006</td>
</tr>
<tr>
<td>Firmness</td>
<td>0,005</td>
</tr>
<tr>
<td>Vitamin C content</td>
<td>0,006</td>
</tr>
<tr>
<td>Color</td>
<td>0,024</td>
</tr>
<tr>
<td>Flavor</td>
<td>0,003</td>
</tr>
<tr>
<td>Appearance</td>
<td>0,008</td>
</tr>
</tbody>
</table>

From this result, we can conclude that:
+ With 12/14 values of columns (a) and (b) smaller than 0,05, we can say that generally both factors affect the studied properties.
+ The effect of concentration of CaCl\(_2\) is more important than dipping time with 6/7 values of column (a) smaller than that of column (b).
+ The interaction of two factors is insignificant with 6/7 values of column (c) greater than 0,05.

These conclusions can be demonstrated visually in Fig. 3. We can recognize that the change of calcium concentration provoke important change in flavor of grape, while variation in dipping time only induce small fluctuation in perception of panelists. From Fig. 3, another observation is that the effect of these factors on properties of grape in preservation is rather complex, we cannot summarize this effect with a simple model.
In comparison 16 treatments, we can realize that the best result (the least changes) is obtained when we use the solution of 1% CaCl$_2$ with 210 seconds of dipping time.

**CONCLUSION**

The experiment show that postharvest treating grape with solution of calcium chloride is an effective method to prolong the shelf life of this commodity. Both 2 factors, the concentration of calcium salt and dipping time, influence significantly the preservation process. Among these factors, the effect of concentration is more important. The experiment also shows that the dipping in solution with 1% of CaCl$_2$ in 210 seconds obtains the best results.

**REFERENCES**


STUDY ON TECHNOLOGICAL PROCESSING OF INSTANT CORN GRUEL

Le Minh Hung, Nguyen Duy Duc, Nguyen Vu Hong Ha
Southern Sub-Institute of Agricultural Engineering and Post Harvest Technology, Hochiminh city, Vietnam; E-mail: minh_hungle@yahoo.com

ABSTRACT
In Vietnam, along with rice, maize (Zea mays indentata L.) cultivation has been fast developed; however, corn is commonly utilized for producing animal feed. Studies of corn-derived food products should be developed to meet increasing demand of export and domestic consumption. A technological processing of instant corn gruel using simple methods basing on freeze-drying principles through a combination of freezing (-18°C in 12 hours) and drying processes (58°C in 3 hours) to produce a high quality food. The mixing of corn and extruded glutinous rice with a fixed ratio (3 corn: 2 rice), was undergone to improve the specific texture of gruel. The small-scale instant corn gruel processing (100kg/batch) using new equipment and technology has been transferred to processors and the instant corn gruel products have been domestically marketed.

Keywords: corn, gruel, freezing, drying, extruding, glutinous rice.

INTRODUCTION
Maize (Zea mays indentata L.) also known as corn is an important food in the global economy ranking first in yield with the annual output of 500 million tons. In Vietnam, corn is grown on 650,000 ha throughout the country in which more than 40% is grown in the northern uplands. Corn ranks with rice as one of the nation’s chief grain crops and its production has increased rapidly by approximately 8.4% annually during the past 10 years due mainly to improved productivities and area expansion (Lam, N. D., 2003). However, since when, corn has been mainly utilized for producing animal feed with just 15 to 20% for human consumption and very few processed corn products have been produce to make the best of corn in term of the availability and high nutritive values.

On the other hand, preservation of corn in Vietnam meets with difficulties since the post harvest handling system is improper and corn will quickly begin to convert sugars into starch after harvest. Even if stored in proper condition in term of optimum temperature and good ventilation, the maximum shelf-life of corn may be only up 4-6 days thus there is a need to process corn in Vietnam (Lien, L.V. et al, 1997). Currently, along with traditional corn food such as corn-sticky rice, corn-cake, boiled and grilled corn, there are some imported but quite expensive products from corn in the markets such as popcorn, canned corn, corn-milk, cornflakes, etc. Therefore, studies on technological process of corn-derived food products will be sound orientation and development to increase value added of corn, make the best of profuse corn source, provide more choices of corn products and increase economic efficiency of corn growers and industry. Through learning about the markets of imported corn products together with Vietnamese food consumption trend, the new product, which is called “Instant corn gruel”, was proposed for studying. The most important point of the study is that corn materials can be freeze-dried by using two simple machines including freezer and drier. This method applied the principle of freeze-drying process which works by freezing the material and then reducing the surrounding pressure and adding enough heat to
allow the frozen water in the material to sublime directly from the solid phase to gas (Fellows, P., 1997). Processing of instant corn gruel does not require large investment and sophisticated machines such as freeze-drier and thus it is useful for low capital food processors.

MATERIALS AND METHODS

Materials

- Corn gruel processed from not too old glutinous corn containing high nutritional values particularly with the amylose pectin content of ≥ 99.2%.
- Rice sample: white glutinous rice Oryza sativa L glutinoza having completely opalescent endoderm with low rate of broken rice (<5%)
- Other ingredients: refined sugar, powder milk, vanilla and coconut flavouring.

Methods

Experiment design

- Experiments were designed to determine optimal parameters in the stages of steaming, freezing, drying, moisturing and mixing.
- Processing stages including steaming, freezing and drying were carried out under several conditions involving different temperature and duration.
- Rice samples were moistened to varying moisture levels to identify their optimal moisture content prior to extrusion cooking
- Mixing rice and corn experiments were carried out to determine the optimal ratio for processing corn gruel.

Sensory analysis

Quality of samples was evaluated based on a taste panel (10 sensory experts) using a 10 points scale (1: worst; 10 best). The quality description involving the desirable aspects in terms of color, texture, taste, aroma, and overall quality was developed in the evaluation sessions.

Physico-chemical and nutritional value analyses

Analyses of physico-chemical, nutritional value and microbiological properties using AOAC and TCVN* (TCVN: Standards of Vietnam) were carried out at the Analysis and Experiment Centre in Hochiminh city. Some physical attributes in terms of texture, hardness, sponginess were analysed by Food Texture Analyser (data processed by TMS-2000 software).
Flowchart of processing of instant corn gruel

- **Corn**
  - Removing husks and silks
  - Washing
  - Steaming
  - Shaving
  - Packing in trays
  - Freezing
  - Drying
  - Screening

- **Glutinous rice**
  - Washing & cleaning
  - Moistening
  - Extruding
  - Grinding/crushing
  - Infrared drying

- **Refined sugar, vanilla, coconut flavour**
  - Weighing
  - Mixing
  - Weighing
  - Packing

- **Instant corn gruel**
RESULTS AND DISCUSSION

Sensory evaluation of instant corn gruel

Products acceptability was evaluated for the sensory attributes in terms of scores. The samples scored above 6.5 in all the 4 quality attributes including texture, colour, aroma and taste indicating highly acceptability or good quality of products.

Factors affecting product’s quality

- **Steaming temperature and duration affecting product quality**

Corn was steamed or boiled and cut off the cob by using a sharp knife. It is important to cut deep enough to get almost the whole kernel, but not so deep to get any of the tip caps attached to the cob that may cause crunchy corn gruel products.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Temp. (°C)</th>
<th>Duration (min.)</th>
<th>Sensory property</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>30</td>
<td>Relatively hard, uncooked</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>30</td>
<td>Soft, well cooked</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>40</td>
<td>Over cooked, relatively doughy</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>30</td>
<td>Doughy, broken</td>
</tr>
</tbody>
</table>

It can be seen in the table 1, the experiment 2 showed best results giving soft and well cooked corn which was steamed at the temperature of 100°C in 30 minutes. The steaming duration is significant to ensure the well cooked corn and to minimise nutrients losses.

- **Temperature and duration of freezing affecting product’s quality**

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Temp (°C)</th>
<th>Duration (hour)</th>
<th>Spongy structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>48</td>
<td>Not spongy</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>24</td>
<td>Less spongy</td>
</tr>
<tr>
<td>3</td>
<td>-5</td>
<td>12</td>
<td>Less spongy</td>
</tr>
<tr>
<td>4</td>
<td>-10</td>
<td>12</td>
<td>Spongy</td>
</tr>
<tr>
<td>5</td>
<td>-18</td>
<td>12</td>
<td>Very spongy</td>
</tr>
</tbody>
</table>

Corn was frozen at the temperature lower than the freezing point of corn fluid and water at which is solidified to ice. The inflexibility of ice water and the stationariness of soluble substances will result in the inactivation of water in corn. Therefore, freezing will minimise nutritional and organoleptic value losses.

Freezing duration varies according to feed types and heat absorbability of materials being frozen. Corn is ordinarily frozen in 12-30 hours. The table 2 showed that the corn being frozen at -18 -> -10 in 12 hours in the experiments 4 and 5 had the best texture of sponginess. The characteristic of spongy structure will help corn be well cooked after adding water to serve.

- **Drying temperature and duration affecting corn’s quality**
Table 3: Drying temperature and duration

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Temp. (°C)</th>
<th>Duration (hour)</th>
<th>Moist. Cont. (%)</th>
<th>Sponginess (%)</th>
<th>Sensory property</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>3</td>
<td>11</td>
<td>70</td>
<td>No corn aroma</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>3</td>
<td>11</td>
<td>80</td>
<td>Aromatic and uniformly dried corn</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>4</td>
<td>10</td>
<td>80</td>
<td>Aromatic and uniformly dried corn</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>3</td>
<td>8</td>
<td>85</td>
<td>Ununiformedly dried corn</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>3</td>
<td>5.5</td>
<td>90</td>
<td>Burnt corn</td>
</tr>
</tbody>
</table>

The moisture content and sponginess of corn gruel and the consistence of well-cooked corn gruel after adding water are critical factors affecting the product’s quality. Drying temperature and duration had to be reduced to the minimum necessary in order to avoid damage of nutrients. On the other hand, drying speed was significantly affecting the sublimation of materials from solids to gas.

The table 3 indicated that corn gruel dried at 60°C in 3 hours in the experiment 2 had the best sensory quality in term of aroma and dryness.

- Moisture content of glutinous rice prior to extrusion

Table 4: Moisture content of glutinous rice prior to extrusion

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Moisture cont. %</th>
<th>Quality of extruded rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>Rice stuck in extruding chamber</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Well cooked &amp; expansive rice</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>Well cooked &amp; expansive rice</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>Hard &amp; uncooked rice</td>
</tr>
</tbody>
</table>

Moisture content of materials is important to extrusion cooking and expansion of extruded rice. Extruding materials in high moisture will produce low temperature and reduce friction among components thereby resulting in unexpensive and uncooked rice. On the contrary, extruding materials in low moisture would produce burnt products (Mercier, C. et al, 1989). The table 4 showed that rice materials of 20-25% would give best extruded rice in term of well-cooked and expansive properties.
Ratio of corn and glutinous rice

Table 5: Mixing ratio of corn and glutinous rice

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Corn (gram)</th>
<th>Glutinous rice (gram)</th>
<th>Quality of gruel added water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>Less corn flavour, no corn colour</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>90</td>
<td>Less corn flavour, no corn colour</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>80</td>
<td>Thickness, not doughiness and corn aroma</td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>70</td>
<td>Less thickness</td>
</tr>
<tr>
<td>5</td>
<td>140</td>
<td>60</td>
<td>Watery state and less thickness</td>
</tr>
</tbody>
</table>

Prepared corn was mixed with extruded glutinous rice at the ratio of 3:2 to produce the specific texture of gruel and enhance the nutritional values of instant corn gruel.

Physico-chemical and nutritional properties

Table 6: Comparison of physico-chemical and nutritional properties between corn gruel and raw corn

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Instant corn gruel</th>
<th>Raw corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>7.3</td>
<td>14</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>7.63</td>
<td>4.6</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>80.64</td>
<td>69.4</td>
</tr>
<tr>
<td>Fiber (%)</td>
<td>0.88</td>
<td>0.4</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Source: The Analysis and Experiment Centre, Hochiminh city

It can be seen in the table 6, the instant corn gruel can be an essentially supplementary food to foster human health and strength due to its high contents of protein and sugar which were 7.63% and 80.64% respectively. Moreover, the instant corn gruel contained high fiber content which was doubled that of the raw corn. This may be useful for those who have difficult digestion and need many meals per day. The low moisture content of 7.3% may help extend the shelflife of the instant corn gruel up to 12 months.

Microbiological property

Table 7: Microbiological property of instant corn gruel

<table>
<thead>
<tr>
<th>No</th>
<th>Criterion</th>
<th>Results</th>
<th>Maximum limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TPC (30°C/72h/g)</td>
<td>7000</td>
<td>$10^4$</td>
</tr>
<tr>
<td>2</td>
<td>Clostridium per. (/g)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Coliform (30°C/24h/g)</td>
<td>&lt; 10</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Total yeast &amp; mould (25°C/5 day/g)</td>
<td>40</td>
<td>$10^2$</td>
</tr>
<tr>
<td>5</td>
<td>E.Coli (/g)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: The Analysis and Experiment Centre, Hochiminh city
The microbiological attributes of instant corn gruel including TPC, Clostridium Perfringens, Coliform, E.Coli, Total Yeast and Mould were within limit and meeting the standards of the Vietnamese Department of Public Health to ensure the hygiene and safety of products.

CONCLUSION
Technological processing of instant corn gruel based on freeze-drying principles was successfully studied to produce spongy and absorbable food products. The instant corn gruel having high nutritive value, good taste, good hygiene and long shelf-life up to 12 months is a healthy food, particularly for children and elderly people. The small-scale instant corn gruel processing (100kg/batch) using the equipment and technology studied has been transferred to some food manufacturers and the instant corn gruel products have been sold and distributed to domestic markets.

REFERENCES


ABSTRACT

Physical characteristics, physicochemical and functional properties, water absorption during soaking, and cooking and eating quality of Thai aromatic rice, Khao Dawk Mali 105 (KDML105) were compared with Khao Hom Changwad (HCW; KDML105, cultivated in Central region of Thailand), Pathum Thani 1 (PTT1; the new breeding aromatic rice) and Chainat 1 (CNT1; the high amylose rice). Cultivation practices showed an effect on cooking and eating quality of KDML105. PTT1, although it was classified in the same type as KDML105, had the different properties and quality from KDML105. High amylose rice (CNT1) was absolutely different from low amylose rice (KDML105) in every properties and quality. The properties and quality of KDML105 have still been the best of rice.

Key Word: KDML105, Thai aromatic rice, physicochemical properties, eating quality

INTRODUCTION

An aromatic rice, KDML105, is considered Thailand’s specialty. It has long grain; once cooked, it permeates a particularly aromatic fragrance, is very soft and chewy, and recently gained wide acceptance.

Due to infertile and drought-striken sandy soils, the cultivation of KDML105 is limited (only 3 - 4 million tons of paddy per year). Therefore, the KDML105 production has been increased and extended to the other parts of Thailand. However, it is known that the best KDML105 production area is in Northeast region of Thailand and the aromatic quality depends on cultivation conditions (Yoshihashi, 2005). Therefore, the aromatic quality of KDML105 cultivated in the other parts of the country is still suspected. Moreover, to increase grain yield of aromatic rice, the new Thai aromatic rice designed to grow as 2nd crop, PTT1 variety, has been bred and produced. The new varieties, however, are not known well and have still been debated on their quality.

Therefore, the aim of this study was to evaluate physical characteristics, physicochemical and functional properties, water absorption during soaking, cooking and eating quality of HCW, PTT1 and CNT1, and compare the properties and quality with original KDML105.

MATERIALS AND METHODS

Three aromatic (KDML105, HCW and PTT1) and one non-aromatic (CNT1) milled rice were used to evaluate the qualities.

An image analyzer was employed to measure length and width of 20 grains of the sample. Size and shape were determined by their greatest dimension and length:width (L/W) ratio, respectively. Degree of whiteness was determined using a whiteness meter (Kett Electric Laboratory, model C-300-3). Weight of 100 grains was measured using a four-decimal balance (Shimadzu, type AX 200).

Amylose content was determined by the method of Juliano (1971) and gel consistency was determined by the method of Cagampang et al. (1973). The method of Little et al. (1958) was used to determine alkali spreading value. The methods reported by Yeh and Yeh (1993); Supprung (2004) were modified to conduct the determination of freeze-thaw stability with 7 freeze-thaw cycles.
The sample (250 g) was soaked in 35°C water and randomly taken to determine the moisture content in every five-minute interval for the period of one hour and every one-hour interval until five hours. The moisture content was determined by the AOAC (1984) method, and the rate of water absorption of milled rice was reported.

Minimum cooking time of the sample was determined by the glass plate-white center method of Ranghino (1969) with slight modification. An optimum cooking time was the minimum cooking time plus two additional minutes. Cooked rice grains of the sample were physically measured using an Image Analyzer. To determine volume expansion, water absorption and residual solids in cooking water, the cooking with excess water method was used.

The sample was cooked by the total absorption cooking method. The amounts of distilled water was determined based on an amylose content of the sample as recommended by Kongseree et al. (2002), W-to-R ratios for low and high amylose rice were 1.7 and 2.4, respectively. For cooked rice texture determination, a texture analyzer (LOLOYD Instrument, model LRX 5K, UK) was used to perform a back extrusion test. The peak extrusion force in kg was determined as a hardness of cooked rice. Sensory analysis of cooked sample was conducted by a descriptive analysis using 10 trained panelists. The concentration of ACPY in the sample was measured using GC-MS with internal standard of TMP. The sensory technique for evaluating the aroma intensities of the cooked sample was rating test, which was modified from Itani et al (2004). Preference test of cooked rice samples was conducted by nine-point hedonic scale, which ranked from nine (like extremely) to one (dislike extremely) using 30 untrained panelists.

RESULTS AND DISCUSSION

Physical Characteristics and Physicochemical Properties
Physical characteristics and physicochemical properties of the samples are presented in Table 1. All the varieties were considered to be long and slender grains since the lengths and L/W ratios were greater than 6.6 mm. and 3.0 as regarded by IRRI (1996). High amylose and non-aromatic rice (CNT1) was markedly higher in size and weight than low amylose and aromatic rice (KDML105, HCW, and PTT1). L/W ratio of KDML105 was the largest and considerably different from the others. The highest whiteness value of CNT1 could be caused by a large amount of chalky grains in this sample.

CNT1 was high amylose rice (>25%) and intermediate gelatinization temperature (GT) while all the others were low (10-19%). Although all the aromatic rice varieties had low amylose, soft gel and low GT, PTT1 was considerably higher in amylose content and lower in gel length and alkali spreading value than the other two. It might be due to the hard texture and separated grain type of the parent line, PTT85061-86-3-2-1.

Water Absorption during Soaking
Figure 1 shows the increases in moisture contents of the rice varieties during soaking in 35°C water for five hrs. The moisture contents increased greatly in the first five min and increased rapidly up to 25-min soaking. After soaking for 5 hrs, the moisture contents of KDML105, HCW, PTT1, and CNT1 were 31.05±0.12, 31.42±0.10, 31.80±0.08, and 27.08±0.25 %db, respectively. It could be concluded that during soaking, low amylose rice absorbed more water than high amylose rice. The moisture content of PTT1 increased faster than that of the others. This observation was related to cooking time of the varieties as discussed later. The faster the water absorbed, the shorter the time required for cooking.
<table>
<thead>
<tr>
<th>Property</th>
<th>Variety</th>
<th>KDML105</th>
<th>HCW</th>
<th>PTT1</th>
<th>CNT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (mm.)</td>
<td>7.26±0.19 b</td>
<td>7.13±0.16 b</td>
<td>6.82±0.17 a</td>
<td>7.38±0.25 c</td>
<td></td>
</tr>
<tr>
<td>Width (mm.)</td>
<td>2.07±0.07 a</td>
<td>2.11±0.07 a</td>
<td>2.07±0.07 a</td>
<td>2.17±0.08 b</td>
<td></td>
</tr>
<tr>
<td>L/W ratio</td>
<td>3.52±0.11 b</td>
<td>3.38±0.13 a</td>
<td>3.30±0.13 a</td>
<td>3.40±0.16 a</td>
<td></td>
</tr>
<tr>
<td>100 grains weight (g)</td>
<td>1.98±0.02 b</td>
<td>1.78±0.02 a</td>
<td>1.82±0.02 a</td>
<td>2.11±0.04 c</td>
<td></td>
</tr>
<tr>
<td>Whiteness (%)</td>
<td>42.20±0.23 a</td>
<td>43.70±0.21 b</td>
<td>42.10±0.21 a</td>
<td>43.80±0.26 b</td>
<td></td>
</tr>
<tr>
<td>Physicochemical properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylose content (%)</td>
<td>16.84±0.07 b</td>
<td>15.98±0.07 a</td>
<td>17.43±0.08 c</td>
<td>28.12±0.08 d</td>
<td></td>
</tr>
<tr>
<td>Gel consistency (mm.)</td>
<td>92.50±2.12 c</td>
<td>90.00±2.83 c</td>
<td>73.00±1.41 b</td>
<td>55.50±2.12 a</td>
<td></td>
</tr>
<tr>
<td>Alkali spreading value</td>
<td>7.00±0.00 b</td>
<td>7.00±0.00 b</td>
<td>6.80±0.42 b</td>
<td>5.00±0.00 a</td>
<td></td>
</tr>
</tbody>
</table>

In a row, means followed by the same letter are not significantly different ($P<0.05$).

**Figure 1.** Changes in moisture content with soaking time of four Thai rice varieties during soaking in 35°C water.

**Freeze-Thaw Stability**

Freeze-thaw stability of the sample pastes (Figure 2) was performed to investigate gel retrogradation. Syneresis of the pastes, except CNT1, increased with increasing freeze-thaw cycle. CNT1 paste showed negligible syneresis along the test. These did not conform to the fact that CNT1, high amylose rice, is retrograded greater than low amylose rice (Kongseree, 2002). The adverse results could be caused by the centrifugal force, which was not enough to release the water from the spongy-like texture of the pastes. However, at the last freeze-thaw cycles, the pastes were compressed between two glass plates (Figure 3). The results showed that the pastes of KDML105 and HCW were still soft and elastic while the pastes of CNT1 having a spongy-like texture already destructed and released a large amount of water. For PTT1, it also had the soft pastes but some of the water in the pastes was squeezed out when the pastes was compressed.
Figure 2. Freeze-thaw stability of four Thai rice varieties

Figure 3. Rice pastes of four Thai rice varieties after treating for 7 freeze-thaw cycles.

Cooking Quality

Optimum cooking time of PTT1 was the lowest (16 min) as shown in Table 2. While KDML105 and HCW needed one more minute than PTT1 for cooking (17 min), CNT1 required the highest time for cooking (21 min). Cooked grain of CNT1 was longer and higher expanded than that of KDML105, HCW, and PTT1, respectively. While percentages of the length expansions of KDML105, HCW, and CNT1 cooked grains were in range of 52-54, that of PTT1 cooked grain was only about 40. Due to the fluffy and swell separate grains of cooked high amylose rice, the volume expansion of cooked CNT1 was the highest. CNT1 exhibited greater absorption of water during cooking and the highest amount of the residual solids in cooking water than the others significantly. The least in residual solids of PTT1 might be due to the cooked rice of PTT1 was very sticky and clumpy, which all of the grains hold together and only a few of residual solids went to the cooking water.
Table 2. Cooking quality of four Thai rice varieties

<table>
<thead>
<tr>
<th>Cooking quality</th>
<th>Variety</th>
<th>KDML105</th>
<th>HCW</th>
<th>PTT1</th>
<th>CNT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum cooking time (min)</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Length (mm.)</td>
<td>11.04±0.35</td>
<td>10.83±0.37</td>
<td>9.60±0.28</td>
<td>11.34±0.29</td>
<td></td>
</tr>
<tr>
<td>Width (mm.)</td>
<td>3.07±0.20</td>
<td>2.87±0.19</td>
<td>2.98±0.15</td>
<td>3.16±0.19</td>
<td></td>
</tr>
<tr>
<td>Length expansion (%)</td>
<td>52.09±0.83</td>
<td>51.94±1.30</td>
<td>40.82±0.62</td>
<td>53.60±0.18</td>
<td></td>
</tr>
<tr>
<td>Width expansion (%)</td>
<td>48.26±1.84</td>
<td>36.16±1.78</td>
<td>43.72±1.09</td>
<td>45.41±1.35</td>
<td></td>
</tr>
<tr>
<td>Volume expansion (ml/ml milled rice)</td>
<td>3.22±0.02 a</td>
<td>3.39±0.04 b</td>
<td>3.18±0.00 a</td>
<td>3.86±0.08 c</td>
<td></td>
</tr>
<tr>
<td>Water absorption (g/g milled rice)</td>
<td>2.62±0.11 a</td>
<td>2.62±0.11 a</td>
<td>2.68±0.08 a</td>
<td>3.08±0.08 b</td>
<td></td>
</tr>
<tr>
<td>Residual solids (%)</td>
<td>1.20±0.09 b</td>
<td>1.22±0.08 b</td>
<td>0.78±0.02 a</td>
<td>1.75±0.10 c</td>
<td></td>
</tr>
</tbody>
</table>

In a row, means followed by the same letter are not significantly different (P<0.05).

Eating Quality

Cooked Rice Texture

At the optimum cooking conditions of each the variety, the value of hardness was the lowest in cooked rice of PTT1 and the highest in cooked rice of HCW. Although CNT1 was high amylose rice, it absorbed a large amount of water enough to get soft texture. Regard to the optimum condition using for cooking KDML105 (W-to-R = 1.7 and minimum cooking time = 15 min), the hardness values of cooked rice of PTT1 and CNT1 were higher than those cooked by their optimum conditions. The hardness value of cooked rice of CNT1 was greatly different from the others and higher than that of HCW, KDML105, and PTT1, respectively.

The sensory intensities of cooked rice of all the varieties are shown in Table 3. Cooked rice of PTT1 was greatly stickier than that of the others and the stickiness intensity of CNT1 was the lowest. While intensity of hardness of CNT1 cooked by its optimum condition was not significantly different from the softest sample (PTT1), this intensity was the highest in CNT1 cooked by the condition of KDML105. The hardness intensity was in accordance with the hardness value obtained from back extrusion test, and the harder the sample was, the less sticky the sample had.

Rice Aroma

The concentration of AcPy in each the variety was conformed to the sensory score for rice aroma (Table 4). The AcPy concentration in KDML105 was the highest and twice as concentrated as PTT1. Although the AcPy concentration in CNT1 was only five times lower than that in KDML105, the aroma score of CNT1 was very low and 30 times lower than that of KDML105. The results showed that the panelists were not able to detect the low concentration of AcPy presenting in CNT1. The aroma quality of KDML105 depends on growth condition; HCW was lower in AcPy concentration and aroma score than KDML105 but the aroma scores of both were not considerably different. It was meant that the panelists were unable to distinguish between aroma quality of KDML105 and HCW.
Table 3. Textural properties of four Thai rice varieties

<table>
<thead>
<tr>
<th>Texture attribute</th>
<th>Variety</th>
<th>KDML105 (1.7, 15)*</th>
<th>HCW (1.7, 15)</th>
<th>PTT1 (1.7, 15)</th>
<th>PTT1 (1.7, 14)</th>
<th>CNT1 (1.7, 15)</th>
<th>CNT1 (2.4, 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Back extrusion test</td>
<td>Hardness</td>
<td>1.82±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.24±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.69±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.15±0.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.68±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Descriptive sensory test</td>
<td>Hardness</td>
<td>7.88±0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.08±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.99±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.72±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.83±0.69&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.87±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Stickiness</td>
<td>8.62±0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.94±0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.61±0.61&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.15±0.73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.62±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.64±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cohesiveness of mass</td>
<td>7.66±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.99±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.09±0.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.09±0.87&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.50±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.51±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Chewiness</td>
<td>47.10±3.14&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>50.10±2.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.10±6.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>42.10±7.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.20±3.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48.20±6.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Toothpack</td>
<td>8.79±0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.94±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.18±0.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.33±0.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.74±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.34±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Loose particles</td>
<td>7.33±0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.31±0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.73±0.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.64±0.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.73±0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.24±0.68&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* (W-to-R ratio, minimum cooking time)

In a row, means followed by the same letter are not significantly different (P<0.05).

Table 4. Concentration of AcPy and score of aroma intensity of four Thai rice varieties

<table>
<thead>
<tr>
<th>Aromatic concentration</th>
<th>Variety</th>
<th>KDML105</th>
<th>HCW</th>
<th>PTT1</th>
<th>CNT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcPy conc. (ng/g)</td>
<td></td>
<td>2200.22</td>
<td>1650.14</td>
<td>1100.09</td>
<td>430.00</td>
</tr>
<tr>
<td>Score for aroma</td>
<td></td>
<td>3.00±0.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.80±0.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.90±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Rating scores: 0 = non-aromatic, 1 = subtle aromatic, 2 = clearly aromatic but not so strong, 3 = aromatic strong as KDML105, and 4 = more aromatic than KDML105

In a row, means followed by the same letter are not significantly different (P<0.05).

Preference of Cooked Rice

Hedonic scores reported for the preference in rice characteristics and qualities are shown in Table 5. Significant differences in all the sensory attributes were observed. Hedonic score of cooked rice of KDML105 was higher than that of the other varieties in all the attributes. The results showed that the sample having small, moist and sticky grains like cooked rice of PTT1 or too dry, hard and fluffy grains like cooked rice of CNT1 were not accepted. The scores in appearance, color, aroma, and overall preference of HCW were also significantly different.
from that of KDML105. It could be explained that the panelists preferred the KDML105 cultivated in Northeast of Thailand than in other areas. Anyway, these results also supported why the consumers prefer KDML105 than others.

Table 5. Hedonic scores of four Thai rice varieties

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Variety</th>
<th>KDML105</th>
<th>HCW</th>
<th>PTT1</th>
<th>CNT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td></td>
<td>7.33±1.09</td>
<td>6.57±1.50</td>
<td>5.77±1.41</td>
<td>6.17±1.70</td>
</tr>
<tr>
<td>Color</td>
<td></td>
<td>7.87±0.63</td>
<td>6.30±1.49</td>
<td>6.30±1.09</td>
<td>7.20±1.03</td>
</tr>
<tr>
<td>Grain separation</td>
<td></td>
<td>6.93±1.01</td>
<td>6.70±1.18</td>
<td>5.47±1.20</td>
<td>5.70±1.49</td>
</tr>
<tr>
<td>Softness</td>
<td></td>
<td>7.00±1.14</td>
<td>6.43±1.55</td>
<td>6.70±1.93</td>
<td>5.80±1.56</td>
</tr>
<tr>
<td>Flavor and taste</td>
<td></td>
<td>6.70±1.06</td>
<td>6.33±1.40</td>
<td>5.53±1.61</td>
<td>5.60±1.52</td>
</tr>
<tr>
<td>Aroma</td>
<td></td>
<td>6.70±1.29</td>
<td>5.90±1.40</td>
<td>5.23±1.68</td>
<td>5.40±1.43</td>
</tr>
<tr>
<td>Overall preference</td>
<td></td>
<td>7.13±0.97</td>
<td>6.40±1.16</td>
<td>5.87±1.20</td>
<td>5.77±1.28</td>
</tr>
</tbody>
</table>

Hedonic score: 1 = dislike extremely, and 9 = like extremely
In a row, means followed by the same letter are not significantly different (P<0.05).

CONCLUSION
Differences in properties and quality of different rice varieties were observed. Comparing HCW with KDML105, cultivation condition obviously affected cooking and eating quality of KDML105. The new breeding aromatic Thai rice variety (PTT1) also had the different properties and quality from KDML105. In addition, rice containing high amylose content (CNT1) showed significant differences in properties and quality from KDML105, which was classified in low amylose type. Among the rice varieties studied, KDML105 was the most preferred.

REFERENCES


SURVIVAL OF FORTIFIED *ESCHERICHIA COLI*, *STAPHYLOCOCCUS AUREUS*, AND *LISTERIA MONOCYTOGENES* IN *NEM CHUA* PRODUCED BY TRADITIONAL TECHNOLOGY AND MODIFIED TECHNOLOGY

*L.T. Truong¹, P. Baumgartner², M.H. Nguyen², J. Markham²*

**ABSTRACT**

*Nem chua* is a traditional fermented sausage popularly consumed in Vietnam. The risk of pathogenic bacterial contamination in the product is high. Modified technology including using starter culture, replacing pounding meat by cutting, packaging in fibrous casings plus vacuum could improve bacterial quality of *nem chua*. Numbers of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* could be reduced to safe levels regulated by laws for consumption.

**INTRODUCTION**

*Nem chua* is a traditionally fermented sausage and popularly consumed in Vietnam. As the product is mostly at small-scaled producers, where hygienic conditions are not strictly controlled, the risk of pathogenic bacteria contaminated into the product is high. Numbers of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* are usually used to contaminate into food products during processing. The presence of these bacteria in food indicates that food is not hygienically produced and a consumption of contaminated food is not safety. Total E. coli in food stuffs should not be detected by laws (at a level of 0 cfu/g for meat). Although food poisoning cases are seldom relating to fermented sausages the risk of infection from nem chua can not be ignored. The use of some commercial starter cultures in combination with modified technologies were proved to inhibit the growth of enterotoxigenic staphylococcus aureus in fermented meat (Gonzalez-Fandos et al., 1994). The aim of this research to study the survival of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* in *Nem Chua* produced by traditional technology and modified technology

**MATERIALS AND METHODS**

**Inoculum preparation:**

*E. coli*, *L. monocytogenes* and *S. aureus* strains were pre-cultured twice in Brain Heart Infusion broth (Oxoid) and incubated at 30°C for 48 h. Serial dilutions were performed with peptone water 0.1% broth to obtain approximately 10^7 cfu/ml. The volumes of cultures fortified into sausage were calculated to obtain an initial quantity of each pathogen of 10^4 viable cells/g.

(1) Total aerobic mesophilic bacteria counts were enumerated following pour plate technique in Plate Count Agar (Oxoid) and incubated at 37 °C for 48 h.

(2) *Staphylococci* and *Staphylococcus aureus* were enumerated via surface plating on Baird Parker Agar (Merck) and incubated at 37 °C for 48 h. Representative colonies with typical black appearance and surrounded by clear zone were picked, and subjected to catalase and coagulase tests (rabbit plasma EDTA, Difco Laboratories, Detroit, Michigan, USA).

(3) Total *E. coli* were analysed according to AOAC Method 46016 (AOAC, 1984).

(6) *L. monocytogenes* according to AOAC Method 46016 (AOAC, 1984).

(7) Total lactic acid bacteria were measured using pour plate technique in MRS (Oxoid) and incubated at 37 °C for 48 h.
Experimental design
Control (traditional technology): Starter culture: nil; packed in plastic bags, fermented at 30°C for 72 h, RH 90%/24 h then 85%/48 h, velocity 0.2-0.3 m/s then stored at 4°C.

Treatment (modified technology): Starter culture CXK (Danisco); stuffed in fibrous casing (P), fermented at 30°C for 48 h, RH 90%/24 h then 85%/24 h, velocity 0.2-0.3 m/s, then stored at 4°C.

RESULTS AND DISCUSSION
The change of pH

![Fig. 3.1 Profile of pH in nem chua produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).](image)

pH value in treatment lots quickly reached to 4.5 after 24 h of fermentation, slightly reduced to about 4.2 in the next 24 h and stabilised at this level from Day 3 to Day 21. In nem chua produced with traditional technology, pH, as usual, reached to 4.5 after 72 h and stabilised at this level up to Day 21.

Change of total plate count, total lactic acid bacteria, and total staphylococci
The count of aerobic bacteria is an indicative parameter of the microbial quality of food products in Vietnam. A high count of aerobic bacteria in food is considered undesirable. The Vietnamese Ministry of Health regulates the level of aerobic bacteria in meat products to be at or less than $3 \times 10^3$ cfu g$^{-1}$ ($= 5.5 \log_{10}$ cfu g$^{-1}$) (Nguyen et al., 2002). Compared to this standard, from Fig. 3.2, the aerobic bacteria in nem chua produced with traditional technologies, which remained high (6.5-7.5 $\log_{10}$ cfu g$^{-1}$) during fermentation and storage periods, was not acceptable. In nem chua produced with modified technologies, it decreased to $5.5 \log$ cfu g$^{-1}$ at day 2, to $4.8 \log_{10}$ cfu g$^{-1}$ at day 7 and then slowly increased to $5.4 \log_{10}$ cfu g$^{-1}$ at day 14. Compared to the Vietnamese standard for aerobic bacteria, nem chua produced with modified technologies was suitable for consumption from day 2 to day 14. It is noted that both nem chua samples were inoculated with potential pathogens. The fairly high number of aerobic bacteria in day 0 of this study is probably due to old pork rind strips having been purchased in the market, although they were still within their use-by date.

In nem chua produced with new technology, number of lactic acid bacteria reached to $10^9$ after 24 h of fermentation and remained unchanged to Day 21 while from Fig. 3.2 the concentration of LAB reached close to $8.8 \log_{10}$ cfu g$^{-1}$ after 24 h of fermentation and remained relatively unchanged to day 21 in nem chua produced with modified technologies,
while it only reached $7.9 \log_{10} \text{cfu g}^{-1}$ after 24 h fermentation, lightly changed for the next 48 h and then slightly reduced to $7.2 \log_{10} \text{cfu g}^{-1}$ at day 21 in the other sample. These data were high compared to the lactic acid counts in the work carried out by Encinas, Sanz, Garcia-Lopez & Otero (1999). The different numbers of lactic acid in *nem chua* produced with traditional technologies and modified technologies might be the main cause of differences of pH level and antimicrobial activity. From Figs 3.2 & 3, it is suggested that an increase of lactic acid production and numbers of LAB probably cause the decrease in aerobic bacteria count.

**Fig 3.2** Profile of aerobic bacteria in *nem chua* produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).

**Fig. 3.3** Profile of LAB in *nem chua* produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).

Although the starter culture was not added into *nem chua* produced with traditional technologies, initial numbers of LAB in *nem chua* produced using two different technologies were not significantly different. However, they varied from day 1 to day 21. The highest concentration of LAB in traditional technologies lots was approximately $8 \log_{10} \text{cfu g}^{-1}$, while the number of LAB in modified technologies lots was about $1 \log_{10} \text{cfu g}^{-1}$ higher from day 1 to day 14. This suggests that not only the initial number but also the species and strains of LAB were important in the success of the meat fermentation process.
Fig. 3.4 shows that the yeast and mould count found in traditional lots was approximately $6 \log_{10} \text{cfu g}^{-1}$ from day 0 to day 21 while in *nem chua* produced with modified technologies the count reduced to $3.6 \log_{10} \text{cfu g}^{-1}$ at day 2 and then increased gradually to $5.2 \log_{10} \text{cfu g}^{-1}$ at day 21. Encinas, Lopez-Diaz, Garcia-Lopez, Otero, & Moreno (2000) found that the increase of pH value in Spanish fermented sausage related to the increase of yeast count due to the consumption of lactic acid by yeast cells. However, in this study, the pH level remained unchanged in *nem chua* produced with modified technologies while the number of yeasts increased from day 3 to day 21. Yeasts and moulds can play an important role in the flavour development of some types of fermented sausages (Lopez Diaz, Gonzalez, Moreno & Otero, 2002; Olesen et al., 2000) but in *nem chua*, their presence is considered as undesirable. The smell described as a “yeasty smell”, caused by metabolites of yeasts and the presence of fungal spots in *nem chua* products, is evaluated as a sign of spoilage, although the limits of yeasts and moulds in meat products are not issued in Vietnam. The growth of yeasts and moulds in *nem chua* could be controlled by an addition of potassium sorbate into *nem chua*; however, this was not addressed in this study.

![Graph](image)

**Fig. 3.4** Profile of yeasts and moulds in *nem chua* produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).

**Survival of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes inoculated in nem chua samples**

The profiles of *E. coli* in *nem chua* produced with traditional technologies and modified technologies are shown in Fig. 3.5. The number of *E. coli* in *nem chua* produced using traditional technologies decreased in the first 24 h but then increased in the next 48 h to $4.5 \log_{10} \text{cfu g}^{-1}$. Finally it reduced slowly to $3.5 \log_{10} \text{cfu g}^{-1}$ at day 21. In *nem chua* produced using the modified technology the number of *E. coli* dropped dramatically after 48 h to $0.3 \log_{10} \text{cfu g}^{-1}$ and remained unchanged until day 21. In Australia, the new microbiological limit in all uncooked comminuted fermented meat is $3.6 \ E. \ coli \ g^{-1}$ (or $0.56 \ log_{10} \ text{cfu g}^{-1}$) (Australian Standard 1.6.1-2006). The requirement of microbiological limit for *E. coli* in Vietnam is $\leq 3 \ E. \ coli \ g^{-1}$ (or $0.48 \ log_{10} \ text{cfu g}^{-1}$) for food products (Nguyen et al., 2002). From the results shown in Fig. 3.5 the concentrations of *E. coli* in *nem chua* produced with modified technology from day 2 to day 21 were lower than both Vietnamese and Australian standards. Although there was a reduction in the first 24 h, the concentrations of *E. coli* in *nem chua* produced with traditional technologies were higher than the limits regulated in the Vietnamese and Australian standards.
Fig. 3.5 Profile of *E. coli* in *nem chua* produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).

From Fig. 3.6, the number of *Staph. aureus* in *nem chua* produced using modified technologies reduced by approximately 1.5 log_{10} cfu g^{-1} in the first 7 days and then remained unchanged until day 21. At the same time, numbers of *Staph. aureus* in *nem chua* produced using traditional technologies increased from about 2 log_{10} cfu g^{-1} from day 0 to day 7 then slightly reduced to 5 log_{10} cfu g^{-1} at day 21. Compared to the Australian Standard 1.6.1 (2006) for UCFM, which is 10^3 cfu g^{-1} or 3 log_{10} cfu g^{-1} coagulase-positive staphylococci, the concentrations of *Staph. aureus* in *nem chua* samples produced with modified technologies were acceptable from day 1 to day 21. However, as the Vietnamese standards for meat products regulate the limits of *Staph. aureus* in meat products to 10 cfu g^{-1} or 1 log_{10} cfu g^{-1} (Nguyen et al, 2002), the concentrations of *Staph. aureus* in *nem chua* inoculated with 10^4 cfu g^{-1} *Staph. aureus* were unacceptable. The Vietnamese standards do not differentiate cooked and uncooked meat products; therefore, in this case, the Australian standard is more realistic for making comparisons. The numbers of *Staph. aureus* in *nem chua* produced with traditional technologies were higher than 3 log_{10} cfu g^{-1} (Australian Standard 1.6.1-2006) for uncooked comminuted fermented meat on every day.

Fig. 3.6 Profile of *Staph. aureus* in *nem chua* produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).
The profiles of *L. monocytogenes* of nem chua produced with traditional technologies and modified technologies are shown in Fig. 3.7.

![Graph showing the profiles of L. monocytogenes](image)

**Fig. 3.7** Profile of *L. monocytogenes* in nem chua produced with traditional technologies and modified technologies. Both samples were inoculated with pathogens. The error bars indicate the SE (n = 6).

The concentrations of *L. monocytogenes* in the first 48 h in nem chua produced using traditional technologies and modified technologies were not significantly different (Fig. 3.7). However, the number of *L. monocytogenes* in modified technology treatment dramatically dropped by day 3 to approximately 1.5 \( \log_{10} \text{cfu g}^{-1} \) in the next 24 h and continued to reduce slightly to lower than 1 \( \log_{10} \text{cfu g}^{-1} \) till day 21. The number of *L. monocytogenes* in nem chua produced using traditional technologies, on the other hand, increased from day 2 to day 4 to the level of 5 \( \log_{10} \text{cfu g}^{-1} \) and remained unchanged in the next 4 days, then slowly reduced to 3.4 \( \log_{10} \text{cfu g}^{-1} \) at day 21. This finding was in agreement with the results reported by Benkerroum, Daoudi and Kamal (2003), who studied the reduction of *L. monocytogenes* in Merguez sausage at the levels of 1.6 and 2.7 \( \log_{10} \text{cfu g}^{-1} \) by bacteriocin-producing starter culture and non-bacteriocin-producing starter culture, respectively. Encinas et al. (1999) found that numbers of *Listeria* spp. in Spanish Chorizo produced without starter cultures were higher than in Chorizo produced with added starter cultures. Similarly, Deumier and Collignan (2003) also reported that chicken dry fermented sausages produced with starter cultures containing more LAB were less contaminated by *L. monocytogenes*. The acceptable limits of *L. monocytogenes* for comminuted unfermented meat are not regulated in Australian Standard 1.6.1 (2006) or for products in Vietnamese standards. However, the recommended limit from the ICMSF (2005) for *L. monocytogenes* in ready-to-eat meat products is 100 cfu g\(^{-1}\) or 2 \( \log_{10} \text{cfu g}^{-1} \). This limit could be obtained in nem chua produced with modified technologies from day 3 to day 21 when the product was inoculated with 4 \( \log_{10} \text{cfu g}^{-1} \) at the processing day (day 0). The low counts of *L. monocytogenes* is probably in relation to the drop of pH and the increase of LAB due to the addition of starter cultures.

From the results in this study, the microbiological quality of nem chua remained high up to day 14, even though the product samples were inoculated with high concentrations of potential pathogens. In addition, no adverse sensory quality of nem chua could be visually found during this period. Therefore the shelf life of nem chua under refrigeration could be set at 14 days.
CONCLUSIONS

The use of modified technologies has been shown to increase the safety of nem chua compared to the use of traditional technologies. The counts of aerobic bacteria, *E. coli* and *Staph. aureus* can reduce to levels lower than the standard limits from high initial counts in nem chua produced with modified technologies. In addition, yeasts and moulds and *L. monocytogenes* counts also reduced significantly, although there are no standard limits for these organisms for this product in Vietnam or Australia. The modified technologies can be implemented for a commercial large scale nem chua production, in keeping with one of the aims of this project. Two main sensory characteristics (colour and texture) of nem chua produced with modified technologies were equal to or better than that of nem chua produced with traditional technologies.

REFERENCES


Considerable progress and technical development can be witnessed in South-East-Asian countries as production of food and feeding the populations is concerned. Unfortunately this statement is only to a certain extent valid for the abattoir sector in SE-Asia and other Asian regions. While countries implemented and realized impressive livestock development programmes, was the production stage of transforming livestock into meat grossly neglected posing serious risks to food safety. In view of that, consumer pressure driven by strong concerns for the safety of meat, is growing stronger on governments demanding to initiate substantial hygienic improvements in the abattoir sector. There is increasing awareness among the public that meat, if not properly treated, is particularly prone to spoilage and can be carrier of zoonotic diseases and food poisoning agents. The recent scares of BSE, Avian Influenza and other zoonotic diseases underscored these sentiments. Even Cholera outbreaks suspected to be caused by contaminated meat are presently reported from the SE-Asian region.

The abattoir sector in SE-Asian countries presents very mixed images and impressions. On the one hand there are a few relatively new, well equipped and hygienically operating abattoirs that produce for export or for domestic quality meat outlets. On the other hand there are large abattoirs in major cities built and equipped longer or shorter periods ago with still functioning core equipment, but mostly in an unsatisfactory state of repair and creating tremendous pollution problems due to their locations mostly in the inner parts of the cities. As a third and largest group exist the high number of small to medium-size private or municipal abattoirs, which present a wide scope of types and categories in terms of availability and quality of equipment and slaughter hygiene, ranging from acceptable slaughter and meat handling procedures to absolutely disastrous and hazardous practices resulting in heavily contaminated meat and serious food safety risks.

The number of slaughterhouses of the traditional type, with slaughter facilities for cattle, buffaloes, small ruminants or pigs is high in SE-Asian countries. The numbers range from 200 to 800 or more in each country, depending on the country size. Comparatively large numbers of such small-scale to medium slaughterhouses are needed, as they cater for the traditional meat markets with “hot” un-refrigerated meat, which requires short delivery ways from the slaughterhouses to the markets. This is the sector where profound technical and hygienic improvements are overdue in order to supply hygienically produced clean meat to consumers. Improvements are urgently required on practically all topics along the meat chain from handling of slaughter animals, slaughtering and carcass dressing as well as meat cutting, boning and meat transports. Severe shortcomings can be registered regarding

- Livestock transports to the slaughterhouses
- Animal welfare issues, in particular malpractices in pre-stunning of slaughter animals.
- Slaughter methods, which in many cases generate high levels of bacterial contamination of the meat due to lack of proper slaughter and by-product handling facilities and careless slaughtering on the part of slaughter personnel.
- Meat cutting, boning and transport frequently done in most unhygienic ways and using inadequate technical and transport facilities.
- Slaughterhouse waste disposal and effluent treatment mostly organized in an unsatisfactory way or not at all, which contributes to the low levels of slaughterhouse hygiene.
- Meat plant cleaning and sanitation mostly incomplete or impossible to be carried out effectively due to the unhygienic structure of premises displaying inadequate building materials, floor and wall cracks and damages as well as heavy corrosion through unavailability of anti-corrosive materials.
- Meat inspection and sanitary control deficient in many slaughterhouses.

**SPECIFIC CASE OF VIETNAM**

**Slaughterhouses for bovines**
Currently there is only one abattoir with line slaughter system for bovines available in the country (VISSAN, HCMC). All other abattoirs for bovines practice floor slaughtering and dressing without any installations to enable the elevation of the carcasses from the floor. The floor area for this type of slaughtering (also called batch or booth slaughtering) gets contaminated with feces and urine of the live animals, followed by contamination through the dirty hides of the animals forced to collapse on the floor, boots or bare feet of the workers and water splashed around washing floor, hides and meat all at the same time. Drained out blood and intestinal content released adds to the contamination.

The carcass surface stripped from its hide is exposed to this contamination. Even worse, it is common practice to completely bone out the carcass on the same spot on the floor thus creating a multitude of new fresh meat surfaces which all get heavily contaminated. To make things even worse, the boneless meat cuts are mostly dragged along the floor to transport vehicles, where they are again deposited on the floor.

The situation in cattle abattoirs is disastrous from the hygienic point of view. It appears that the grave hygienic deficiencies in cattle abattoirs with floor slaughtering are now recognized by some major meat entrepreneurs in cooperation with the local veterinary authorities, as there are currently moves on the way to construct three new abattoirs for the meat supply of HCMC and one for Hanoi containing line slaughtering for cattle using proven imported equipment. Similarly, one private cattle slaughter entrepreneur in HCMC province plans to change the inadequate floor slaughtering system currently in operation to line slaughtering. Technical assistance should be given in this case to avoid construction and installation failures, as it is obviously intended to have the equipment fabricated by local constructors of steel equipment, who most probably lack sufficient experience in the abattoir sector.

**Slaughterhouses for pigs**

Different types of slaughterhouses for pigs are available. Some of them are without any technical installations. Even the scalding is done by pouring hot water on the skin of the carcasses. Slaughtering, splitting and carcass cutting has to be carried out on the floor with the same disastrous consequences as described above for comparable cattle operations. Other pig abattoirs have semi-line systems with suspension on overhead rails of the pigs after scalding and scraping. Such systems go along with considerable hygienic improvements, as at hygienically critical stages of slaughtering and dressing such as head removal and opening of the body cavities, where meat surfaces can get exposed to possible contamination, the carcasses are off the floor and suspended in vertical position.

One small pig abattoir exists in HCMC that can be considered a model facility for full-line slaughtering. Amongst others it was developed through technical inputs by a FAO co-sponsored project. Pigs are mechanically elevated into the scalding tank and scraping machine and onto the dressing rail without floor contact. Interestingly, the electrically heated scalding vat, electrically driven scraping machine and also the electrical stunning tongs were designed
by the mentioned project and locally built by using high-quality stainless steel at the Engineering Department of Nom Lang University (formerly UAF/University for Agriculture and Forestry HCMC). The model scraping machine has already been replicated and one unit is in use in one of the semi-line pig slaughter facilities mentioned above.

These are very good developments and hopefully followed-up further for the upgrading of the pig slaughter sector, which is of outstanding importance in Vietnam. As pig slaughtering is technically less demanding than cattle slaughtering, some hygienically satisfactory premises are used. However, it appears that the majority of pig slaughter facilities in the country are technically and hygienically obsolete and urgent improvements are needed.

One critical area is the pig scalding, where in the scalding vats too small amounts of water are used for too many pigs put through. Renewal of water should be effected frequently. Moreover, there is no control of the scalding water temperature, which ideally should be in the range of 62°C, but in reality is mostly too hot even up to boiling point. Excessively high scalding temperatures will damage the skin through protein denaturizing and cause skin lesions through scraping and can even have a negative impact on meat quality (PSE meat or other biochemical alterations).

Another critical area is the pig stunning with in Vietnam “home-made” wooden electric stunning tongs. Mostly no transformer is used but the electrical current from the mains applied. This can be a torture for the pigs and also have a negative impact on the meat quality. Some other hygienic issues such as inserting the hooks in the Achilles tendon and not as usual in pigs in the cut-free tendons of the hind foot or not careful enough hair removal through mechanical or manual scraping are still common and should be rectified.

**Abattoir development**

In Vietnam the partly opening-up to market economy in the 80ies and 90ies was favourable for general economic progress but caused, similar to other SE-Asian countries, very unfavourable developments in the slaughterhouse sector. Free market economy triggered the mushrooming of many small private slaughter places, mostly with obsolete technical and hygienic installations or practically none at all. These small slaughterhouses run without much cost for maintenance, hygiene measures and energy could operate cheaper than the large central abattoirs and took the major part of slaughter animals away from the established large ones. The consequence was that butchers slaughtered cheaper, but slaughter hygiene in general declined dramatically and sanitary control was difficult or could not be implemented at all.

Due to the still strong government presence in Vietnam’s economy, which also puts the veterinary authorities in a comparable stronger position than in many other Asian countries, Vietnam’s veterinary service managed to reverse the above described development.

One should expect that under the sometimes hygienically absolute disastrous conditions encountered in slaughterhouses of the region, meat spoilage and severe problems to consumers’ health, amongst them serious illnesses caused by Salmonella or entero-pathogenic E. coli, would be wide-spread. Obviously this is not the case at a large scale, but the threat exists and presumably many cases occur or other cases may not be traced down to meat where the problem originated. The tendency in the traditional meat marketing system to keep the meat delivery chain as short as possible from the abattoir through the market to the consumer is certainly also a means to keep economic and public health damages at bay. Most efficient and averting major health problems are the Asian cooking habits, which traditionally apply intensive heat treatment and where meat is cut into small pieces thus making sure that all tissue parts are sufficiently heated. However, this system only works satisfactorily to some extend, as long as the meat contaminating microorganisms do not have much chance to develop further because of the short periods from slaughterhouse to the consumer.
Presently changes of the meat marketing systems can be witnessed in many developing countries. While formerly all meat was marketed traditionally, namely un-refrigerated in open markets, sales outlets for chilled meat are emerging in particular in population centers. There is an increasing tendency in Asian countries – in some more and in some less pronounced yet – to produce high quality chilled meat for domestic sales. Such productions, from a tiny fraction to estimated 15% of the overall meat market depending on the country, are handled by the private sector and there is awareness that only technically high standard and hygienic abattoirs with refrigeration units can supply such markets. However, in some instances outlets for pre-packed chilled meat such as supermarkets, because of lack of suitable meat sources from hygienic slaughter facilities, are still supplied with the hygienically sub-standard “hot” meat from traditional abattoirs. This is a very dangerous practice as meat in supermarkets or other quality meat shops undergoes prolonged storage periods and there is a high risk of massive growth of spoilage and food poisoning bacteria if the raw material was already heavily contaminated as it is usually the case with meat from traditional abattoirs. Such practices can prove hazardous to consumers and must be discontinued. Meat to be sold chilled by supermarkets and quality meat shops must undergo refrigeration at the abattoir immediately after slaughter.

Also globalization in meat trade has encompassed developing countries, who either receive certain types of meat through imports or some countries even participate actively in global meat trade by producing meat from certain livestock species for export. These developments demand profound changes in the way livestock is slaughtered in particular in the SE-Asian countries, amongst them Vietnam.

As already mentioned, Vietnam, through various modern abattoir projects, appears to be on a good way in improving the hygienic conditions for meat to be supplied to the consumer. However, there is still a long way to go to improve the abattoir situation countrywide. Still the large majority of private and public slaughterhouses cater for the traditional meat markets with “hot” unchilled meat.

This is the sector where profound technical and hygienic improvements are needed in order to supply hygienically produced clean meat to consumers. Improvements are urgently required on practically all topics along the chain of handling of slaughter animals, slaughtering and carcass dressing as well as meat cutting and boning.

It has to be realized that traditional slaughter systems cannot be changed in the short term in most SE-Asian countries, as these are an integrated part of the traditional meat marketing system. Traditional meat marketing systems are a result of the simple infrastructure prevailing in the meat sector in many developing regions. The urgent improvement of the traditional slaughter systems is therefore needed as an immediate measure. However, it should not be lost view of desirable future developments, which are countrywide systems of hygienically run abattoirs with efficient installations and applying uninterrupted cold chains.

Hereunder, some principles for the upgrading of the abattoir sector are given:

**Abattoirs for bovines**

Bovine slaughter facilities are designed either according to the *booth/batch slaughter system* or the *line slaughter system*. For larger capacity abattoirs simultaneous booth or batch slaughtering of a certain number (“batch”) of bovines, each animal at one designed spot (“booth”) on the slaughter floor, is strongly discouraged due to a multitude of hygienic problems arising during such procedures. Booth slaughtering (Fig. 1) is solely justified in case of slaughtering one or a very small number of bovines, where congestion on the slaughter floor and careless working habits can be avoided.
Hereunder, recommendations are given and examples drawn up on how the traditional abattoir sector can be improved.

Slaughtering of bovines

In principle cattle slaughterhouses should apply the line slaughter system, which is the commonly accepted and preferred technology. Simultaneous booth or batch slaughtering of a certain number (“batch”) of bovines, each animal at one designed spot (“booth”) on the slaughter floor, is strongly discouraged due to a multitude of hygienic problems arising during such procedures. Booth slaughtering (see Fig. 1) is solely justified in case of slaughtering one or a very small number of bovines, where congestion on the slaughter floor and careless working habits can be avoided.

In Fig. 1 the sequence of the slaughter and dressing operations in the booth slaughter system are shown. Operations a1 to a5 are carried out in the same booth. The manual lifting facility shown is a wall attached hoist, which in this case is preferable to a simple manual chain block. Instead of manual hoists, electric hoists can be used, which facilitate the butchers work. If a skinning cradle is used the hoist of the booth slaughter system has to be employed two times, first for lifting up the carcass and lowering it again for placement on the skinning cradle. The second step, where the hoist is needed when utilizing a skinning cradle, is the lifting up of the partly flayed carcass to vertical position for completion of flaying as well as for evisceration and splitting.

For all medium to larger scale bovine slaughtering the line slaughter system is strongly recommended. Line slaughter systems for bovines can be subdivided into simplified line slaughtering also called semi-line slaughtering, and continuous line slaughtering (Fig. 2). The difference between the two systems is that in continuous line slaughtering the carcass, once hoisted up after stunning, remains for the entire process in vertical position on the line while semi-line slaughtering uses vertical and horizontal positions. The horizontal operations take place with the carcass on a skinning cradle. For this purpose the carcass has to be lowered from its vertical position to the horizontal position on the cradle, where the initial flaying operation starts and feet are cut. Having done this, the carcass is gradually lifted up until landed in vertical position on the rail for complete flaying, evisceration and splitting.

In the continuous line slaughtering system (Fig. 2), the animal is hoisted up for bleeding and the carcass remains in this position during the entire flaying and dressing process. In continuous line slaughtering two rails with different heights, the bleeding rail (4.50 m) and the dressing rail (3.50 m), are needed. The change of hooks takes place at the transfer station(Fig. 2).
Abattoirs for pigs

In pig slaughtering the guiding principle should be that, after removal of the hair, there should be no further contact of the pig skin (which is food) with the floor of the slaughter premises.

In medium and larger pig slaughter operations, which are the common type in most Asian countries, hygienic slaughtering can easily be achieved by equipping the slaughter premises with complete railing systems, electrically heated scalding vats, lifting facilities into and out of the scalding vat, dehairing machines with mechanical loading and unloading devices, elevation device onto the rail and electrical splitting saw. This is the modern outfit for a pig slaughter line (see Fig. 3).

However, in most pig slaughter premises in SE-Asian countries, slaughter lines of the described type are not available and not likely to be introduced soon. Such investments may even not be necessary at this stage as long as most of the pork goes un-refrigerated to the markets and consumers.

Great advantages in working conditions and meat hygiene in still simple and only basically mechanized pig slaughtering can be achieved, if slaughtering and dressing is carried out on slaughter floors, which are built at different levels (“terrace system”). In the terrace system, the killing of the pigs takes place at the highest floor level and carcasses are gradually passed during the various slaughter steps to the lower floor levels. This system utilizes the forces of gravity and hence facilitates moving the carcasses without complicated electro-mechanical elevation equipment (Fig. 4).
The overhead railing in this simple but hygienic pig slaughter system starts at the scraping/gambreling area. From here all operations can be organized in a straightforward and hygienic manner. The rail installed above the scraping table for carcass suspension by using gambrels can be kept at a low height. This greatly facilitates the manual lifting up to the rail of the gambrels with the hind legs of the pigs hooked in. The operators for the next operations, evisceration and splitting, are positioned at another lower “terrace” floor level and can conveniently carry out their tasks, as the height of the rail at their working place is approx. 2.60 m. There is no risk of the carcasses getting in contact with the floor at this rail height.

This described system does not require high investment cost, is not prone to mechanical failures and allows reasonably hygienic slaughtering. It can therefore be recommended for medium-scale operations, where technically more sophisticated systems can not (yet) be afforded.
APPLICATION OF MPN - MPN (MOST PROBABLE NUMBER - POLYMERASE CHAIN REACTION) TO ENUMERATE Clostridium botulinum SPORES IN HONEY

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2, Institute of Applied Microbiology, Georg-August-University Goettingen, Germany

ABSTRACT

Scientific studies have recognised the great medical value of honey. However, Clostridium botulinum (C. botulinum) spores have been found in honey that was implicated in infant botulism, a neuroparalytic disease caused by a neurotoxin produced in the infant’s intestine after spores of these bacteria are ingested and grow.

The objective of the present study was to develop a MPN-PCR procedure (Most Probable Number - Polymerase Chain Reaction) to enumerate spores of different types of C. botulinum A, B, C, D, E, and F in spiked honey samples by using an enrichment followed by a PCR procedure, targeting the neurotoxin genes. Sterilized honey samples were spiked with a known amount of spores of different strains of C. botulinum, with each strain separately and a mixture of all the strains.

The enumeration of spores was carried out by applying MPN-PCR method. Two media, FAB (Fastidious Anaerobe Broth) and CMM (Cooked Meat Medium), were used as enrichment media. The numbers of individual strain and mixed strain spores in the spiked samples were similar to the number of spiked spores. The results were similar between the two enrichment media. It can be concluded that the applied method can be used to enumerate C. botulinum spores in honey samples despite the high sugar concentration. The method is time-saving and avoids using animals.

Keywords: C. botulinum, enumeration, honey, infant botulism, MPN-PCR, spores

INTRODUCTION

C. botulinum includes seven groups of anaerobic spore forming bacteria producing extremely potent botulinum neurotoxins (Cato et al., 1986). The neurotoxins cause botulism, a paralytic condition in man and animals. Infant botulism is caused by ingestion of C. botulinum spores and results in colonisation of the intestinal tract and botulinum toxin production (CDC, 1998).

To date, honey has been regarded as one of foodstuffs being a significant risk factor for infant botulism. C. botulinum spores have been detected in honey samples in the United States, Europe, Japan, and Brazil. Cases of infant botulism with a history of honey consumption have been reported.

Most Probable Number method (MPN), which involves enrichment in a liquid medium and a mouse bioassay (MBA), is frequently used to enumerate C. botulinum spores in foods (FDA, 1992). This method is time consuming and requires experimental animals. The use of the MPN method followed by PCR has been widely investigated with the aim of reducing time consumption and avoiding ethical concerns.

Objective

Enumeration of spores of these strains in spiked honey samples using the MPN-PCR method, targeting the neurotoxin encoding genes.
MATERIALS AND METHODS

Bacterial strains
Spores of *C. botulinum* types A, C, D, E and proteolytic types B and F.

Media
Cooked Meat Medium (CMM, DIFCO, Detroit) and Fastidious Anaerobe Broth (FAB)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Meat peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3g</td>
</tr>
<tr>
<td>Dextrose (D-Glucose monohydrate)</td>
<td>1g</td>
</tr>
<tr>
<td>Solube starch</td>
<td>1g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5g</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>1g</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.4 g</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride</td>
<td>0.5 g</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>1g</td>
</tr>
<tr>
<td>Trizma base</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Haemin</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Vitamine K1</td>
<td>0.001 g</td>
</tr>
<tr>
<td>Sodium succinate</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>14g</td>
</tr>
<tr>
<td>Destilled water</td>
<td>ad 1000 ml</td>
</tr>
</tbody>
</table>

pH 7.2 ± 0.2 at 25 °C, autoclaved at 121 °C for 15 min. Haemin, L-Arginine, trizma base, and sodium bicarbonate (SIGMA-ALDRICH, Germany). Other ingredients (DIFCO, Detroit).

Preparation of spiked honey samples
Honey was purchased from supermarkets and sterilised by autoclaving it at 115°C for 30 min. After sterilisation, the honey was streaked onto blood agar plates and also put into FAB tubes. This was incubated aerobically and anaerobically for 3-7 days for examination of sterility. A known amount of individual spores of each *C. botulinum* type A, B, C, D, E, or F was added to these honey samples. In addition, other honey samples were inoculated with a mixture of all strains containing equal numbers of spores of each strain. Triplicate samples of inoculated honey were prepared.

Enumeration procedure
The enumeration of *C. botulinum* spores in spore suspensions and in spiked honey was determined using the five-tube-MPN method in CMM and FAB. This was confirmed by mouse bioassay and PCR.

Series of 5 tubes containing 4.5 mL of CMM and FAB were inoculated with 0.5 mL of spore suspension or 0.5 g honey. This was considered as the 10¹ dilution. For further dilutions spore suspension and honey samples were 10-fold serially diluted in sterile water. From each dilution, 0.5 mL was transferred to 5 tubes containing 4.5 mL of enrichment media. For spore suspension, duplicate test samples were tested in FAB, while for honey samples CMM and
FAB were used in parallel to determine which medium was better for recovery of *C. botulinum* spores. All tubes were heated at 60°C for 30 min, and then cooled immediately with cold water. The number of spores in suspension of each strain was obtained after an anaerobic incubation for 4 days at 35-37°C. Tubes showing bacterial growth were checked with PCR and MBA. The number of *C. botulinum* spores was obtained by converting the number of positive tubes to a MPN from the reference table (FDA, 1992).

**RESULTS AND DISCUSSION**

**Enumeration of *C. botulinum* spores in spiked honey samples**

There was significant difference between the spore number of types C and D (p<0.05). Significant differences between type D and types B and F; and between type C and types A and E were observed. No significant difference in the number of types A and E as well as types B and F. Type D revealed the highest recovery, while type C the lowest recovery.

In honey inoculated with mixed spores of the *C. botulinum* types, *C. botulinum* type E revealed the highest, whereas type D the lowest number. There was a significant difference in MPN between the two types. No significant difference (p<0.05) was obtained in the number of types A, B, C, and F.

The number of recovered spores of *C. botulinum* estimated by MPN-PCR was similar to, or higher than number of spiked spores (Fig. 1). Similar results were obtained in honey spiked with mixed spore types except for types B and D recovery rates were lower.

The confirmation using MBA was carried out with growing tubes at the highest dilution. Similar results were obtained in comparison to that in PCR procedure.

Comparison of MPN of *C. botulinum* in honey samples inoculated with individual (group A) and mixed (group B) types of spores between the two enrichment media FAB and CMM was carried out. In the both media, no difference in the number of *C. botulinum* in honey samples of group A as well as group B was estimated by MPN-PCR (Table 2 and table 3). The two enrichment media CMM and FAB revealed no significant difference in MPN of *C. botulinum* types A to F per gram of honey.
Fig. 1: Comparison of recovery of *C. botulinum* in inoculated honey with individual spore types in different media.
Fig. 2: Comparison of recovery of *C. botulinum* in honey inoculated with mixed spore types in different media
Table 2: Recovery of *C. botulinum* types A to F in honey samples inoculated with individual type of spores from different enrichment media

<table>
<thead>
<tr>
<th>Medium</th>
<th>n</th>
<th>MPN (PER G)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>CMM</td>
<td>18</td>
<td>83,278&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68,212</td>
</tr>
<tr>
<td>FAB</td>
<td>18</td>
<td>78,056&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47,959</td>
</tr>
</tbody>
</table>

*n, number of samples; <sup>a</sup>Means with the same superscripts are not significantly different (p>0.05)*

Table 3: Recovery of *C. botulinum* in honey samples spiked with mixed types of spores from different enrichment media

<table>
<thead>
<tr>
<th>Media</th>
<th>n</th>
<th>MPN (per g)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>CMM</td>
<td>18</td>
<td>2,339&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,181</td>
</tr>
<tr>
<td>FAB</td>
<td>18</td>
<td>2,613&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,802</td>
</tr>
</tbody>
</table>

*n, number of samples; <sup>a</sup>Means with superscripts are not significantly different (p>0.05)*

**CONCLUSION**

The applied MPN-PCR method can be used to enumerate *C. botulinum* spores in honey despite the high concentration of sugar in honey. Revealing the similar recovery yields, CMM and FAB were equally suitable for recovery of *C. botulinum* as enrichment media. The MPN-PCR method is obviously more convenient and reliable, and avoids ethical issues using experimental animals.

**Acknowledgements**

We gratefully thank Friedrich-Ebert-Stiftung and the Institute of Applied Microbiology, Georg-August-University Göttingen for financial and technological support.

**REFERENCES**


STUDY ON DETERMINING HARVESTING INDEXES OF SOME SPECIFIC FRUITS IN ORDER TO IMPROVE THEIR QUALITY IN PRESERVATION AND PROCESSING

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ABSTRACT

Harvesting time of fruits has been so far identified by farmers’ experience and sensibility instead of reliably scientific data. The quality of harvested fruits, therefore, is low and unstable resulting in quick deterioration, short shelf life and increasing losses of fruits. These problems are considered as the main cause of decreasing economic output of farmers. The 3 year-study on determining harvesting indexes of specific fruits in Tien Giang, Ben Tre, Dong Thap, Vinh Long, Binh Duong and Ho Chi Minh city in order to improve fruit quality in preservation and processing has been conducted by the Southern-Sub- Institute of Agricultural Engineering & Post harvest Technology. The results obtained indicated the proper harvesting time (from after flowering to harvesting), respiration rate, sensory characteristics (appearance, colour, odour, taste, texture…), physico-chemical properties (TSS, moisture content, glucid, lipid, organic acid, Vitamin C…) and other specifications (hardness, skin thickness, links of fruit & peduncle…) of six different kinds of fruit, such as: ‘Lo Ren” star-apple, “Sugar” mandarin, “Yellow Pulp & Dwindled Seed” durian, “Sanh” orange, “Green Skin” pomelo and mangosteen.

The fruits in this study are often used for fresh consuming so “Sugar” mandarin should be harvested during 221th-227th day after early fruiting (AEF), “Sanh” orange should be harvested from 212th to 221th day AEF, “Green Skin” pomelo should be harvested during 210th-220th day AEF, “Lo Ren” star-apple should be harvested at 240th-244th day AEF, “Yellow Pulp & Dwindled Seed” durian should be harvested at 113th-118th day AEF and mangosteen should be harvested at level: 5,6,7.

The results also indicated the proper harvesting time for each of six fruits according to its consuming purposes: fresh consumption, processing and storage.

Key works: harvesting, indexes, after flowering, sensory, physico-chemical

INTRODUCTION

Located in the tropical and temperate zone, southern provinces are more likely to favourably develop agricultural production in term of area specializzing in growing fruits. According to statistic of the Nationally Agricultural Extension Bureau, the fruit cultivation area in southern provinces reach 393,140 ha over the total 747,800ha of the whole country.

Harvesting time of fruits has been so far identified by farmers’ experience and sensibility instead of reliably scientific data. Fruits harvested, therefore, are immature and low quality or overripe and quickly deteriorated resulting in either short shelf life and uncontrolled losses of fruits or loss of prestige to consumers. Harvesting fruits at correct time is significant to improve fresh quality, to extend self-life and to enhance quality of further processed fruits. Many agriculturally developed countries, such as Australia, France, Thailand… have studied for a long while past on determining harvest time which might be the very first beginning of either storing or processing a fruit. Not yet research on harvesting time of crops in Vietnam, particularly in fruit has been done to help farmers deal with their crop’s problems in term of
short self-life, low quality and safety. Handbooks, manuals, training guides and scientific data on harvesting time of fruit should be recommended to be identified and published for farmers. The 3 year-study on determining harvesting indexes of specific fruits in Tien Giang, Ben Tre, Dong Thap, Vinh Long, Binh Duong and Ho Chi Minh city in order to improve fruit quality in preservation and processing, therefore, has been conducted by the Southern-Sub- Institute of Agricultural Engineering & Post harvest Technology. The six kinds of fruits have been studied are as follows: “Sugar” mandarin, “Sanh” orange, “Green Skin” pomelo ‘Lo Ren” star-apple, “Yellow Pulp & Dwindled Seed” durian and mangosteen.

MATERIALS AND METHODS

Materials

The six kinds of fruits, such as: ‘Lo Ren” star-apple, “Sugar” mandarin, “Yellow Pulp & Dwindled Seed” durian, “Sanh” orange, “Green Skin” pomelo and mangosteen were studied and monitored from flowering to harvesting stages at different orchards in Tien Giang, Ben Tre, Dong Thap, Vinh Long, Binh Duong and Ho Chi Minh city.

Methods

Identification of proper practice of marking flowers on field

- During the flowering stage, flowers were randomly marked according to diagonal rule in the orchards from East to West and from bottom to top of the tree.
- Flowers were marked twice or thrice repetitively in each season of the year at certain orchards.
- Determination of the number of flowers marked each time, the age of tree and the experimenting area for specific fruits were also carried out.
- Either main and secondary seasons or harvesting stages of fruits were identified and evaluated. Since each fruit has its own metabolism and growth rate
- The sampling frequency and sampling time of these six fruits in the study varied from fruit to fruit and were determined according to the duration of sampling and after flowering or early fruiting.

Determination of the most correct harvesting duration:

Determination of the most correct harvesting duration was carried out by calculating the number of days from early fruiting to harvesting according to sensory, physio-chemical properties and respiration rate of fruits.

Determining respiration rate of six kinds of fruit

1. **Respiration rate**

   Respiration rate (RR) was determined by the continuous air flow at specific temperature in 24 hours using automatic CO₂ analyzer. The CO₂ concentration was determined by the following formula:

   \[
   RR (\text{mgCO}_2/\text{kg/h}) = \frac{(C1-C0) \times L \times 60 \times 273 \times 44 \times 1000}{100 \times 22.4 \times (t_0 + 273) \times m}
   \]

   - \(C1\) : CO₂ concentration of sample (%)
   - \(C0\) : CO₂ concentration of the current atmosphere (%)
   - \(L\) : Air flow (ml/minute) through sample.
   - \(273\) : Kelvin temperature (°K)
   - \(t0\) : Ambient temperature (°C)
   - \(44\) : Molecular weight of CO₂ (gram).
   - \(22.4\) : Standard molecular volume (l).
Analyses of physico-chemical properties of six kinds of fruit

1. **Weight of fruit**: determined by scale (g or kg)
2. **Colour index**
   Change of fruit colour was assessed by colourimetric device as Minotal CR-200 (L, a*, b*)
   a*: green to red (-60 -- +60)
   b*: blue to yellow (-60 -- +60)
3. **Hardness of fruit**: Fruit hardness (kg/cm²) was measured by penetrometer
4. **TSS (Brix)**: TTS was measured by digital refractometer.
5. **TA**: Total acidity (organic acidity) was determined by measurement of titration with NaOH 0,1N using phenolphthalein 1% as indicator.
6. **Vitamin C**: Vitamin C content was determined by measurement of titration with 2,6 diclophenol -indophenol 0,001%.
7. **Flesh/fruit ratio**:
   \[ T(\%) = \frac{m1 \times 100}{m} \]
   T: Ratio (%), m: fruit weight (g), m1: flesh weight (g).
8. **Lipid content (%):**
   Total lipid content was determined by taking ether extraction by Soxhlet apparatus whereas percentage of lipid was determined by calculating initial and final weight of samples.
   Total lipid content was measured as the following formula:
   \[ X\% = \frac{(G1-G2) \times 100}{G} \]
   G: weight of sample (g).
   G1: weight of lipid and cup (g).
   G2: weight of empty cup (g).
9. **Glucid content (%):**
   Total glucid indicated as glucose or inverted sugar in 100g was measured as following formula:
   \[ X = \frac{(G1 \times 100) \times \text{Dilution degree}}{(G \times 1000)} \]
   G1: weight of inverted sugar or glucose (mg) equivalent to KMnO₄ 0,1N.
   G: Initial weight of sample (g)
   1000: convert from mg to g.

**Sensory evaluation of six kinds of fruit according to Australian standard**

<table>
<thead>
<tr>
<th>Sensoty (taste, flavour)</th>
<th>Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely delicious</td>
<td>9</td>
</tr>
<tr>
<td>Very delicious</td>
<td>8</td>
</tr>
<tr>
<td>Delicious</td>
<td>7</td>
</tr>
<tr>
<td>Rather delicious</td>
<td>6</td>
</tr>
<tr>
<td>Average</td>
<td>5</td>
</tr>
<tr>
<td>Rather bad</td>
<td>4</td>
</tr>
<tr>
<td>Bad</td>
<td>3</td>
</tr>
<tr>
<td>Very bad</td>
<td>2</td>
</tr>
<tr>
<td>Terrible</td>
<td>1</td>
</tr>
</tbody>
</table>
RESULTS & DISCUSSION

Correct harvest time of six kinds of fruit

The fruits in this study are often used for fresh consuming so they should be harvested when reaching the maximum level of quality and likely to be instantly eaten. According to table 1,2,3,4,5,6 & 7, “Sugar” mandarin should be harvested during 221th -227th day after early fruiting (AEF), “Sanh” orange should be harvested from 212th to 221th day AEF, “Green Skin” pomelo should be harvested during 210th -220th day AEF, “Lo Ren” star-apple should be harvested at 240th-244th day AEF, “Yellow Pulp & Dwindled Seed” durian should be harvested at 113th-118th day AEF and mangosteen should be harvested at level: 5,6,7.

Respiration rate and physico-chemical properties of six kinds of fruit:

Fruit harvesting time varies according to its consuming purposes: fresh, processing and storage. For fresh consumption, fruits should reach the maximum of quality in terms of best sensory property and highest contents of glucid, TSS, TA, Vit.C, juice... and optimum level of respiration rate.

The physico-chemical properties and sensory characteristics of six different kinds of fruit including ‘Lo Ren” star-apple, “Sugar” mandarin, “Yellow Pulp & Dwindled Seed” durian, “Sanh” orange, “Green Skin” pomelo and mangosteen also were indicated in table 1,2,3,4,5,6 and 7. “Sugar” mandarin, “Sanh” orange, “Green Skin” pomelo and ‘Lo Ren” star-apple are nonclimacteric fruits of which the natural storage is longer and post-harvest change is not so dramatic, therefore, their respiration rates were steadily decreased during ripening stage. The flesh/fruit ratio, TSS/TA ratio, glucid, Vit C of “Sugar” mandarin, “Sanh” orange, “Green Skin” pomelo and ‘Lo Ren” star-apple had a gradual increase while the their acidity was decreased after early fruiting. Unlike citrus and ‘Lo Ren” star-apple, “Yellow Pulp & Dwindled Seed” durian and mangosteen are climacteric fruits which are allowed to keep on ripening after harvest. The glucid and lipid contents of “Yellow Pulp & Dwindled Seed” durian and the acidity and Vit.C of mangosteen as well as the flesh/fruit ratio and TSS of both “Yellow Pulp & Dwindled Seed” durian and mangosteen showed an stable increase. In the case of climacteric fruits, it is suggested to harvest physiologically immature fruits to extend their self-life. If fruit is too immature, however, it might not ripen to a good eating quality. Sensory characteristics of six different fruits harvested at the correct time are presented.

Sensory characteristics of six kinds of fruit studied

“Sugar” mandarin

- Appearance: thin, shiny skin and succulent fruit. The middle bottom of fruit is sunken along with the inflated skin around stem may help mandarin skin be easily removed from the flesh.
- Colour: green-yellow skin
- Flavour: slightly sour and quite sweet, and not bitter afterwards.

Harvest maturity of fruit is significant to its quality and shelf-life. “Sugar” mandarin early harvested may result in sour taste and bitter flavour afterwards while those late harvested may cause weight loss, dry flesh, yellow turning skin, insipid taste.

“Sugar” mandarin is often fresh consumed so should be harvested during 221th -227th day

‘Sanh’ orange

- Appearance: shiny peel and easily removed peel from flesh, a little hole (1.5-2mm in diameter) at the middle bottom of orange.
- Colour: green or light yellow colour peel
- Flavour: from less sour to quite sweet.
“Sanh” orange early harvested gave dark green colour and resulted in sour taste and bitter flavour afterwards. Like mandarin, ‘Sanh’ orange is often used for fresh consuming so should be harvested from 212th to 221th day after early fruiting according to table 2.

“Green Skin” pomelo
- Appearance: seedless, waterish/juicy flesh, lumpy skin particularly the soft skin of middle bottom can be tested by using gently finger pressing.
- Colour: pink colour flesh
- Flavour: sweet, less sour

“Green Skin” pomelo early harvested may result in hard bottom of fruit and seedy pomelo while late harvest may cause odourless and dry, less juicy, hard flesh. “Green Skin” pomelo is often fresh consumed so should be harvested during 210th -220th day after early fruiting according to table 3.

“Lo Ren” star-apple
- Appearance: succulent and shiny skin. Particularly, stem is easily removed from body
- Colour: pink colour at middle bottom of skin
- Flavour: sweet, milky taste, less sap

“Lo Ren” star-apple early harvested gave green colour skin, insipid taste, more sap and less edible portions of fruit. Those late harvested may result in tasteless, dark pink and brown pomelo along with high sensibility to abrasion and damage during harvesting, transportation and storage due to their thin skin.

The results indicated that the stage 2 gave highest quality star-apple and ”Lo Ren” star-apple should be harvested at 240th-244th day after early fruiting as can be seen in the table 4. It is stated that star-apple might be rapidly ripe and remarkably varying after 238 days from early fruiting, therefore, regularly monitoring should be taken care to harvest the most properly mature mangosteen.

“Yellow Pulp & Dwindled Seed” durian
- Appearance: sharply pointed spines of skin form a brown, zigzag and uninterrupted track from top to bottom of mature durian. The track location makes a sign of where the compartments containing segments are and therefore, durian skin can be easily cut and removed. It is noted that this track is only occurred on mature durians and once identified, peeling durian become much more easy. Stem is easily removed from durian body. Durian flesh is soft.
- Colour: creamy-white or yellowish coloured flesh
- Odour: specific aroma of durian and powerful odour
- Flavour: buttery taste and nutty flavour

According to results analysed, “Yellow Pulp & Dwindled Seed” durian is a climateric fruit, which might be allowed to continuously ripen after harvest. However, harvesting too immature durian should not be recommended due to the negative impact on sensory characteristic of durian in terms of insipid and no buttery taste. In case of late harvest of durian, the skin is easily removed during harvesting and transporting resulting in soft and flaccid flesh of durian. “Yellow Pulp & Dwindled Seed” durian is often fresh consumed so should be harvested at 113th-118th day after early fruiting.
Mangosteen

Unlike other fruits, harvesting time for mangosteen was clearly determined by skin colour. The maturity degrees of mangosteen were divided into 7 levels based on colour changes of the skin. The results obtained are as follows:

- Fresh mangosteen for near market: at level 5, 6, 7
- Fresh mangosteen for long-distance market: at level 3, 4
- Mangosteen for storage: at level 1, 2, 3, 4.

In comparison to Lai Thieu mangosteens, it took longer time to ripen Cai Mon ones which had thicker peel and sourer taste. However, a problem popularly occurred to Cai Mon mangosteen, particularly in rainy season, is the yellow sap discharge which may cause hardness and inedibility as well as reduce the mangosteen flesh quality and lower farmer’s income. In spite of the long season of mangosteen, its price at the end season is often low since the sap discharge has been occurred. Scientists have put many efforts to overcome this impediment but satisfactory results have not yet been successfully achieved.
Table 1. Technical standards for monitoring quality according to maturity change of “Sugar” mandarin at the main season (March-Dec)

<table>
<thead>
<tr>
<th>Early fruiting time Criteria</th>
<th>195</th>
<th>200</th>
<th>205</th>
<th>210</th>
<th>213</th>
<th>215</th>
<th>218</th>
<th>221</th>
<th>224</th>
<th>227</th>
<th>230</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate (mgCO2/kg/h)</td>
<td>22.54</td>
<td>27.69</td>
<td>25.88</td>
<td>26.40</td>
<td>24.42</td>
<td>23.79</td>
<td>20.30</td>
<td>21.73</td>
<td>21.96</td>
<td>24.29</td>
<td>20.73</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>58.9</td>
<td>59.4</td>
<td>61.2</td>
<td>62.1</td>
<td>62.7</td>
<td>63.5</td>
<td>64.0</td>
<td>64.1</td>
<td>64.3</td>
<td>64.9</td>
<td>65.1</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>102.43</td>
<td>104.97</td>
<td>106.66</td>
<td>118.66</td>
<td>120.09</td>
<td>120.33</td>
<td>120.61</td>
<td>120.82</td>
<td>120.95</td>
<td>121.05</td>
<td>121.07</td>
</tr>
<tr>
<td>Skin colour L(*)</td>
<td>36.87</td>
<td>37.22</td>
<td>38.51</td>
<td>39.10</td>
<td>40.02</td>
<td>42.17</td>
<td>43.87</td>
<td>44.92</td>
<td>46.41</td>
<td>46.85</td>
<td>47.09</td>
</tr>
<tr>
<td>Skin colour b(*)</td>
<td>25.62</td>
<td>26.12</td>
<td>27.89</td>
<td>28.63</td>
<td>30.11</td>
<td>33.15</td>
<td>35.71</td>
<td>39.23</td>
<td>43.70</td>
<td>45.21</td>
<td>47.56</td>
</tr>
<tr>
<td>Ratio of flesh/fruit (%)</td>
<td>78.21</td>
<td>78.69</td>
<td>79.12</td>
<td>80.11</td>
<td>81.00</td>
<td>81.62</td>
<td>81.9</td>
<td>82.67</td>
<td>83.20</td>
<td>83.51</td>
<td>83.80</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>10.0</td>
<td>10.3</td>
<td>10.5</td>
<td>10.8</td>
<td>11.2</td>
<td>11.5</td>
<td>12.0</td>
<td>12.1</td>
<td>12.3</td>
<td>11.7</td>
<td>11.2</td>
</tr>
<tr>
<td>TA (%)</td>
<td>0.321</td>
<td>0.332</td>
<td>0.316</td>
<td>0.291</td>
<td>0.275</td>
<td>0.251</td>
<td>0.230</td>
<td>0.227</td>
<td>0.221</td>
<td>0.214</td>
<td>0.212</td>
</tr>
<tr>
<td>TSS/TA</td>
<td>31.15</td>
<td>31.02</td>
<td>33.23</td>
<td>37.11</td>
<td>40.73</td>
<td>45.82</td>
<td>52.17</td>
<td>53.30</td>
<td>55.66</td>
<td>54.67</td>
<td>52.83</td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td>29.54</td>
<td>30.90</td>
<td>30.98</td>
<td>31.50</td>
<td>32.39</td>
<td>32.42</td>
<td>32.67</td>
<td>32.88</td>
<td>33.36</td>
<td>33.83</td>
<td>33.52</td>
</tr>
<tr>
<td>Juice (%)</td>
<td>73.31</td>
<td>73.53</td>
<td>74.16</td>
<td>75.51</td>
<td>76.23</td>
<td>77.61</td>
<td>78.13</td>
<td>79.50</td>
<td>80.81</td>
<td>80.56</td>
<td>80.25</td>
</tr>
<tr>
<td>Hardness (kg/cm²)</td>
<td>3.92</td>
<td>3.65</td>
<td>3.01</td>
<td>2.65</td>
<td>1.98</td>
<td>1.45</td>
<td>1.34</td>
<td>1.21</td>
<td>1.11</td>
<td>1.02</td>
<td>1.04</td>
</tr>
<tr>
<td>Sensory test (marks)</td>
<td>5</td>
<td>5.5</td>
<td>6.0</td>
<td>6.5</td>
<td>7.0</td>
<td>7.5</td>
<td>8.0</td>
<td>8.5</td>
<td>9.0</td>
<td>9.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Note: TSS: Total Soluble Solid; TA:Total Acidity; L: 0 ÷ 100 (Light); a: -60 ÷ 60 (Green ÷ Red); b: -60 ÷ 60 (Blue ÷ Yellow).

“Sugar” mandarin is harvested at 2 seasons: main (March-Dec) and secondary season (Apr, May -Feb, Mar). Only main reason is presented in this report.
Table 2. Technical standards for monitoring quality according to maturity change of ‘Sanh’ orange at the main season (Jan-July)

<table>
<thead>
<tr>
<th>Early fruiting time Criteria</th>
<th>190</th>
<th>195</th>
<th>200</th>
<th>203</th>
<th>206</th>
<th>209</th>
<th>212</th>
<th>215</th>
<th>218</th>
<th>221</th>
<th>224</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate (mgCO₂/kg/h) (**)</td>
<td>28.94</td>
<td>28.54</td>
<td>28.25</td>
<td>27.70</td>
<td>25.76</td>
<td>22.90</td>
<td>20.66</td>
<td>19.01</td>
<td>18.95</td>
<td>17.21</td>
<td>16.45</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>74.7</td>
<td>75.5</td>
<td>77.3</td>
<td>78.5</td>
<td>79.1</td>
<td>79.8</td>
<td>80.6</td>
<td>80.9</td>
<td>81.3</td>
<td>81.7</td>
<td>81.9</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>198.12</td>
<td>207.13</td>
<td>218.39</td>
<td>225.35</td>
<td>229.27</td>
<td>232.56</td>
<td>236.24</td>
<td>239.65</td>
<td>241.89</td>
<td>243.78</td>
<td>243.53</td>
</tr>
<tr>
<td>Skin thickness (mm)</td>
<td>3.78</td>
<td>3.72</td>
<td>3.63</td>
<td>3.51</td>
<td>3.47</td>
<td>3.40</td>
<td>3.38</td>
<td>3.35</td>
<td>3.30</td>
<td>3.28</td>
<td>3.26</td>
</tr>
<tr>
<td>Skin colour L(*)</td>
<td>27.37</td>
<td>28.65</td>
<td>29.18</td>
<td>30.64</td>
<td>32.70</td>
<td>33.99</td>
<td>35.87</td>
<td>38.15</td>
<td>46.98</td>
<td>47.73</td>
<td>49.13</td>
</tr>
<tr>
<td>B(*)</td>
<td>1.05</td>
<td>3.13</td>
<td>5.45</td>
<td>8.72</td>
<td>12.55</td>
<td>14.26</td>
<td>15.44</td>
<td>18.88</td>
<td>22.47</td>
<td>28.46</td>
<td>30.44</td>
</tr>
<tr>
<td>Ratio of flesh/fruit (%)</td>
<td>69.61</td>
<td>70.11</td>
<td>71.23</td>
<td>72.35</td>
<td>73.17</td>
<td>74.26</td>
<td>75.53</td>
<td>77.51</td>
<td>78.69</td>
<td>79.70</td>
<td>80.22</td>
</tr>
<tr>
<td>Total sugar (%)</td>
<td>6.55</td>
<td>6.75</td>
<td>6.91</td>
<td>7.04</td>
<td>7.11</td>
<td>7.20</td>
<td>7.55</td>
<td>7.72</td>
<td>8.01</td>
<td>8.24</td>
<td>8.32</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>7.1</td>
<td>7.5</td>
<td>7.8</td>
<td>8.1</td>
<td>8.6</td>
<td>8.9</td>
<td>9.3</td>
<td>10.0</td>
<td>10.3</td>
<td>10.4</td>
<td>10.2</td>
</tr>
<tr>
<td>TA (%)</td>
<td>2.98</td>
<td>2.82</td>
<td>2.76</td>
<td>1.99</td>
<td>1.19</td>
<td>0.85</td>
<td>0.65</td>
<td>0.63</td>
<td>0.62</td>
<td>0.61</td>
<td>0.59</td>
</tr>
<tr>
<td>TSS/TA</td>
<td>2.38</td>
<td>2.66</td>
<td>2.82</td>
<td>4.07</td>
<td>7.23</td>
<td>10.47</td>
<td>14.31</td>
<td>15.87</td>
<td>16.61</td>
<td>17.05</td>
<td>17.29</td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td>11.73</td>
<td>12.52</td>
<td>13.05</td>
<td>13.95</td>
<td>14.89</td>
<td>15.54</td>
<td>16.10</td>
<td>17.69</td>
<td>18.15</td>
<td>18.09</td>
<td>17.98</td>
</tr>
<tr>
<td>Juice (%)</td>
<td>65.28</td>
<td>67.11</td>
<td>68.22</td>
<td>69.87</td>
<td>70.43</td>
<td>71.65</td>
<td>72.61</td>
<td>73.71</td>
<td>74.80</td>
<td>75.14</td>
<td>74.10</td>
</tr>
<tr>
<td>Hardness(kg/cm²)</td>
<td>4.71</td>
<td>4.10</td>
<td>3.45</td>
<td>2.68</td>
<td>2.12</td>
<td>1.93</td>
<td>1.68</td>
<td>1.50</td>
<td>1.42</td>
<td>1.35</td>
<td>1.22</td>
</tr>
<tr>
<td>Sensory test (marks)</td>
<td>3.5</td>
<td>4</td>
<td>4.5</td>
<td>5</td>
<td>5.5</td>
<td>7.0</td>
<td>8.5</td>
<td>9.0</td>
<td>9.0</td>
<td>8.5</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Note: ‘Sanh’ orange is harvested at 2 seasons: main (Jan-July) and secondary season (Oct-May). Only main reason is presented in this report.
Table 3. Technical standards for monitoring quality according to maturity change of “Green Skin” pomelo at the main season

<table>
<thead>
<tr>
<th>time Criteria</th>
<th>Early fruiting</th>
<th>174</th>
<th>181</th>
<th>188</th>
<th>195</th>
<th>202</th>
<th>207</th>
<th>210</th>
<th>213</th>
<th>216</th>
<th>219</th>
<th>222</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate (mgCO₂/kg/h)</td>
<td>53.85</td>
<td>53.17</td>
<td>52.88</td>
<td>48.04</td>
<td>42.71</td>
<td>30.73</td>
<td><strong>25.11</strong></td>
<td><strong>23.26</strong></td>
<td><strong>22.71</strong></td>
<td><strong>22.13</strong></td>
<td>20.98</td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>171.2</td>
<td>187.7</td>
<td>192.3</td>
<td>209.0</td>
<td>213.1</td>
<td>226.8</td>
<td><strong>232.2</strong></td>
<td><strong>234.2</strong></td>
<td><strong>236.0</strong></td>
<td><strong>237.6</strong></td>
<td>238.1</td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1.67</td>
<td>1.83</td>
<td>2.01</td>
<td>2.12</td>
<td>2.23</td>
<td>2.31</td>
<td><strong>2.38</strong></td>
<td><strong>2.42</strong></td>
<td><strong>2.51</strong></td>
<td><strong>2.59</strong></td>
<td>2.63</td>
<td></td>
</tr>
<tr>
<td>Skin thickness (mm)</td>
<td>3.60</td>
<td>3.21</td>
<td>3.10</td>
<td>2.85</td>
<td>2.60</td>
<td>2.50</td>
<td><strong>2.41</strong></td>
<td><strong>2.25</strong></td>
<td><strong>2.18</strong></td>
<td><strong>2.12</strong></td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>Skin colour</td>
<td><strong>L</strong>(*)</td>
<td>29.03</td>
<td>29.81</td>
<td>30.50</td>
<td>32.69</td>
<td>34.82</td>
<td>35.87</td>
<td><strong>37.04</strong></td>
<td><strong>41.01</strong></td>
<td><strong>48.52</strong></td>
<td><strong>49.63</strong></td>
<td>50.61</td>
</tr>
<tr>
<td></td>
<td><strong>A</strong>(*)</td>
<td>-13.90</td>
<td>-12.25</td>
<td>-10.41</td>
<td>-6.03</td>
<td>-5.27</td>
<td>-3.20</td>
<td><strong>2.03</strong></td>
<td><strong>3.51</strong></td>
<td><strong>4.93</strong></td>
<td><strong>6.81</strong></td>
<td>7.21</td>
</tr>
<tr>
<td></td>
<td><strong>B</strong>(*)</td>
<td>1.75</td>
<td>2.56</td>
<td>3.81</td>
<td>8.28</td>
<td>11.14</td>
<td>13.05</td>
<td><strong>14.31</strong></td>
<td><strong>15.62</strong></td>
<td><strong>17.71</strong></td>
<td><strong>18.01</strong></td>
<td>20.96</td>
</tr>
<tr>
<td>Ratio of flesh/fruit (%)</td>
<td>55.10</td>
<td>59.77</td>
<td>65.39</td>
<td>70.62</td>
<td>74.97</td>
<td>76.87</td>
<td><strong>78.46</strong></td>
<td><strong>79.25</strong></td>
<td><strong>79.59</strong></td>
<td><strong>79.68</strong></td>
<td>79.82</td>
<td></td>
</tr>
<tr>
<td>Total sugar (%)</td>
<td>6.36</td>
<td>6.51</td>
<td>6.90</td>
<td>7.22</td>
<td>7.50</td>
<td>7.71</td>
<td><strong>7.90</strong></td>
<td><strong>8.02</strong></td>
<td><strong>8.15</strong></td>
<td><strong>8.22</strong></td>
<td>8.20</td>
<td></td>
</tr>
<tr>
<td>TSS (%)</td>
<td>8.00</td>
<td>8.55</td>
<td>9.00</td>
<td>9.47</td>
<td>9.67</td>
<td>10.00</td>
<td><strong>10.37</strong></td>
<td><strong>10.45</strong></td>
<td><strong>10.87</strong></td>
<td><strong>10.93</strong></td>
<td>10.87</td>
<td></td>
</tr>
<tr>
<td>TA (%)</td>
<td>0.931</td>
<td>0.823</td>
<td>0.686</td>
<td>0.584</td>
<td>0.446</td>
<td>0.418</td>
<td><strong>0.368</strong></td>
<td><strong>0.350</strong></td>
<td><strong>0.330</strong></td>
<td><strong>0.247</strong></td>
<td>0.238</td>
<td></td>
</tr>
<tr>
<td>TSS/TA</td>
<td>8.59</td>
<td>10.39</td>
<td>13.11</td>
<td>16.22</td>
<td>21.68</td>
<td>23.92</td>
<td><strong>28.18</strong></td>
<td><strong>29.86</strong></td>
<td><strong>32.94</strong></td>
<td><strong>44.25</strong></td>
<td>45.67</td>
<td></td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td>38.10</td>
<td>40.87</td>
<td>43.49</td>
<td>44.93</td>
<td>46.05</td>
<td>46.97</td>
<td><strong>47.41</strong></td>
<td><strong>48.06</strong></td>
<td><strong>48.26</strong></td>
<td><strong>48.43</strong></td>
<td>47.27</td>
<td></td>
</tr>
<tr>
<td>Juice (%)</td>
<td>32.21</td>
<td>35.45</td>
<td>48.27</td>
<td>52.67</td>
<td>54.95</td>
<td>55.32</td>
<td><strong>56.67</strong></td>
<td><strong>58.73</strong></td>
<td><strong>59.01</strong></td>
<td><strong>59.78</strong></td>
<td>58.45</td>
<td></td>
</tr>
<tr>
<td>Hardness(kg/cm²)</td>
<td>11.01</td>
<td>10.62</td>
<td>9.41</td>
<td>9.01</td>
<td>8.30</td>
<td>7.12</td>
<td><strong>6.84</strong></td>
<td><strong>6.60</strong></td>
<td><strong>6.43</strong></td>
<td><strong>6.31</strong></td>
<td>6.27</td>
<td></td>
</tr>
<tr>
<td>Sensory test (marks)</td>
<td>4.0</td>
<td>4.5</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td><strong>8.5</strong></td>
<td><strong>9.0</strong></td>
<td><strong>9.0</strong></td>
<td><strong>8.5</strong></td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Harvesting time of “Green Skin” pomelo might be divided into 2 stages: stage 1 (Jun-Dec), stage 2 (Jan-July). Only stage 1 is presented in this report.
Table 4. Technical standards for monitoring quality according to maturity change of “Lo Ren” star-apple at the stage 2 (Mar-Dec)

<table>
<thead>
<tr>
<th>time Criteria</th>
<th>Early fruited</th>
<th>190</th>
<th>195</th>
<th>200</th>
<th>203</th>
<th>206</th>
<th>209</th>
<th>212</th>
<th>215</th>
<th>218</th>
<th>221</th>
<th>224</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate (mgCO₂/kg/h)</td>
<td>(*)</td>
<td>28.17</td>
<td>28.04</td>
<td>27.01</td>
<td>26.82</td>
<td>24.13</td>
<td>20.53</td>
<td>19.66</td>
<td>18.91</td>
<td>18.43</td>
<td>17.15</td>
<td>16.23</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td></td>
<td>73.5</td>
<td>74.7</td>
<td>76.4</td>
<td>77.0</td>
<td>77.6</td>
<td>78.3</td>
<td>78.7</td>
<td>79.0</td>
<td>79.3</td>
<td>79.9</td>
<td>80.1</td>
</tr>
<tr>
<td>Weight (g)</td>
<td></td>
<td>186.77</td>
<td>195.70</td>
<td>200.74</td>
<td>202.17</td>
<td>205.02</td>
<td>209.13</td>
<td>211.20</td>
<td>214.28</td>
<td>218.22</td>
<td>220.15</td>
<td>220.21</td>
</tr>
<tr>
<td>Skin thickness (mm)</td>
<td></td>
<td>3.82</td>
<td>3.75</td>
<td>3.68</td>
<td>3.59</td>
<td>3.50</td>
<td>3.46</td>
<td>3.41</td>
<td>3.37</td>
<td>3.34</td>
<td>3.30</td>
<td>3.28</td>
</tr>
<tr>
<td>Skin colour L(*)</td>
<td></td>
<td>28.15</td>
<td>28.98</td>
<td>29.71</td>
<td>31.45</td>
<td>33.81</td>
<td>34.87</td>
<td>36.90</td>
<td>39.24</td>
<td>47.18</td>
<td>48.82</td>
<td>50.80</td>
</tr>
<tr>
<td>Skin colour a(*)</td>
<td></td>
<td>-13.60</td>
<td>-12.81</td>
<td>-10.27</td>
<td>-6.82</td>
<td>-5.13</td>
<td>-3.91</td>
<td>3.71</td>
<td>4.70</td>
<td>5.92</td>
<td>7.97</td>
<td>9.04</td>
</tr>
<tr>
<td>Skin colour b(*)</td>
<td></td>
<td>1.71</td>
<td>4.32</td>
<td>6.70</td>
<td>9.91</td>
<td>14.01</td>
<td>16.75</td>
<td>17.98</td>
<td>19.90</td>
<td>24.63</td>
<td>29.94</td>
<td>31.15</td>
</tr>
<tr>
<td>Ratio of flesh/fruit (%)</td>
<td></td>
<td>69.10</td>
<td>70.03</td>
<td>70.38</td>
<td>71.20</td>
<td>72.17</td>
<td>73.95</td>
<td>75.11</td>
<td>77.04</td>
<td>78.14</td>
<td>79.23</td>
<td>80.01</td>
</tr>
<tr>
<td>Total sugar (%)</td>
<td></td>
<td>6.10</td>
<td>6.14</td>
<td>6.31</td>
<td>6.78</td>
<td>7.01</td>
<td>7.15</td>
<td>7.31</td>
<td>7.45</td>
<td>7.92</td>
<td>8.02</td>
<td>8.13</td>
</tr>
<tr>
<td>TSS (%)</td>
<td></td>
<td>6.7</td>
<td>6.9</td>
<td>7.1</td>
<td>7.5</td>
<td>8.0</td>
<td>8.3</td>
<td>9.1</td>
<td>10.0</td>
<td>10.1</td>
<td>10.2</td>
<td>10.0</td>
</tr>
<tr>
<td>TA (%)</td>
<td></td>
<td>2.85</td>
<td>2.69</td>
<td>2.61</td>
<td>1.86</td>
<td>1.17</td>
<td>0.83</td>
<td>0.70</td>
<td>0.65</td>
<td>0.61</td>
<td>0.60</td>
<td>0.57</td>
</tr>
<tr>
<td>TSS/TA</td>
<td></td>
<td>2.35</td>
<td>2.56</td>
<td>2.72</td>
<td>4.03</td>
<td>6.34</td>
<td>10.00</td>
<td>13.00</td>
<td>15.38</td>
<td>16.56</td>
<td>17.00</td>
<td>17.54</td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td></td>
<td>11.10</td>
<td>11.24</td>
<td>12.37</td>
<td>13.03</td>
<td>13.71</td>
<td>14.01</td>
<td>15.46</td>
<td>16.93</td>
<td>17.72</td>
<td>17.51</td>
<td>17.13</td>
</tr>
<tr>
<td>Juice (%)</td>
<td></td>
<td>63.11</td>
<td>64.29</td>
<td>65.16</td>
<td>66.28</td>
<td>67.11</td>
<td>68.54</td>
<td>69.22</td>
<td>69.96</td>
<td>70.61</td>
<td>71.01</td>
<td>70.26</td>
</tr>
<tr>
<td>Hardness (kg/cm²)</td>
<td></td>
<td>4.80</td>
<td>4.36</td>
<td>3.80</td>
<td>3.03</td>
<td>2.81</td>
<td>2.01</td>
<td>1.75</td>
<td>1.61</td>
<td>1.48</td>
<td>1.40</td>
<td>1.34</td>
</tr>
<tr>
<td>Sensory test (marks)</td>
<td></td>
<td>3.5</td>
<td>4.4</td>
<td>4.5</td>
<td>5.5</td>
<td>7.0</td>
<td>7.5</td>
<td>8.0</td>
<td>8.5</td>
<td>8.5</td>
<td>8.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Note: Harvesting time of “Lo Ren” star-apple might be divided into 3 stages: stage 1 (Feb-Nov), stage 2 (Mar-Dec) and stage 3 (Apr, May-Jan, Feb). Only stage 2 is presented in this report.
Table 5. Technical standards for monitoring quality according to maturity change of “Yellow Pulp & Dwindled Seed” durian at stage I (Jan-Apr, May)

<table>
<thead>
<tr>
<th>Early fruiting time</th>
<th>85</th>
<th>90</th>
<th>95</th>
<th>100</th>
<th>105</th>
<th>108</th>
<th>110</th>
<th>113</th>
<th>115</th>
<th>118</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate(*) (mgCO₂/kg/h)</td>
<td>98.17</td>
<td>99.26</td>
<td>101.12</td>
<td>109.45</td>
<td>215.20</td>
<td>224.76</td>
<td>538.39</td>
<td>447.12</td>
<td>394.83</td>
<td>325.76</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>1.91</td>
<td>2.16</td>
<td>2.24</td>
<td>2.32</td>
<td>2.54</td>
<td>2.70</td>
<td>2.75</td>
<td>2.81</td>
<td>2.83</td>
<td>2.85</td>
</tr>
<tr>
<td>Skin colour L(*)</td>
<td>72.19</td>
<td>75.51</td>
<td>77.30</td>
<td>80.95</td>
<td>82.74</td>
<td>86.54</td>
<td>89.09</td>
<td>89.45</td>
<td>89.82</td>
<td>89.80</td>
</tr>
<tr>
<td>Skin colour a(*)</td>
<td>-13.26</td>
<td>-10.12</td>
<td>-8.41</td>
<td>-4.05</td>
<td>-3.45</td>
<td>-2.75</td>
<td>-1.86</td>
<td>1.12</td>
<td>1.45</td>
<td>2.50</td>
</tr>
<tr>
<td>Skin colour b(*)</td>
<td>26.39</td>
<td>27.11</td>
<td>28.20</td>
<td>30.29</td>
<td>34.23</td>
<td>41.81</td>
<td>43.01</td>
<td>46.85</td>
<td>48.08</td>
<td>48.76</td>
</tr>
<tr>
<td>Glucid (%)</td>
<td>26.21</td>
<td>27.13</td>
<td>27.59</td>
<td>28.11</td>
<td>28.57</td>
<td>29.32</td>
<td>29.89</td>
<td>30.31</td>
<td>31.86</td>
<td>32.56</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>7.5</td>
<td>8.1</td>
<td>9.6</td>
<td>10.5</td>
<td>11.8</td>
<td>15.5</td>
<td>20.1</td>
<td>22.8</td>
<td>25.4</td>
<td>25.9</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>2.15</td>
<td>2.99</td>
<td>3.22</td>
<td>3.72</td>
<td>4.69</td>
<td>4.93</td>
<td>5.27</td>
<td>5.45</td>
<td>5.70</td>
<td>5.81</td>
</tr>
<tr>
<td>Flesh hardness (kg/cm²)</td>
<td>11.21</td>
<td>10.43</td>
<td>8.23</td>
<td>7.31</td>
<td>5.85</td>
<td>4.23</td>
<td>2.10</td>
<td>1.53</td>
<td>0.73</td>
<td>0.51</td>
</tr>
<tr>
<td>Sensory test (marks)</td>
<td>2.0</td>
<td>2.5</td>
<td>3.5</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.5</td>
<td>9.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

**Note:** Harvesting time of durian might be divided into 3 stages: stage 1 (Jan-Apr, May), stage 2 (Feb-Jun) and stage 3 (Mar-Jul). Only one stage is presented in this report.
Table 6. Technical standards for monitoring quality according to maturity change of “Cai Mon” Mangosteen (Ben Tre).

<table>
<thead>
<tr>
<th>Maturity Criteria</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate (mgCO₂/kg/h)</td>
<td>154.78</td>
<td>145.75</td>
<td>132.89</td>
<td>126.34</td>
<td>121.23</td>
<td>100.21</td>
<td>98.65</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>13.2</td>
<td>15.5</td>
<td>16.0</td>
<td>16.2</td>
<td>16.3</td>
<td>16.7</td>
<td>17.2</td>
</tr>
<tr>
<td>TA (%)</td>
<td>0.572</td>
<td>0.767</td>
<td>0.764</td>
<td>0.810</td>
<td>0.828</td>
<td>0.771</td>
<td>0.698</td>
</tr>
<tr>
<td>TSS/TA</td>
<td>23.08</td>
<td>20.21</td>
<td>20.94</td>
<td>20.00</td>
<td>19.69</td>
<td>21.66</td>
<td>24.64</td>
</tr>
<tr>
<td>Glucid (%)</td>
<td>5.36</td>
<td>6.72</td>
<td>7.11</td>
<td>7.25</td>
<td>8.19</td>
<td>8.63</td>
<td>8.30</td>
</tr>
<tr>
<td>Ratio of flesh/fruit (%)</td>
<td>45.22</td>
<td>48.78</td>
<td>52.54</td>
<td>58.77</td>
<td>62.10</td>
<td>70.51</td>
<td>72.36</td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td>4.898</td>
<td>4.603</td>
<td>4.683</td>
<td>5.104</td>
<td>5.154</td>
<td>5.471</td>
<td>5.214</td>
</tr>
<tr>
<td>Skin hardness (%kg/cm²)</td>
<td>12.5</td>
<td>10.8</td>
<td>8.2</td>
<td>6.0</td>
<td>3.0</td>
<td>2.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Sensory Test</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8.5</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Table 7. Technical standards for monitoring quality according to maturity change of “Lai Thieu” Mangosteen (Binh Duong).

<table>
<thead>
<tr>
<th>Maturity Criteria</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate (mgCO₂/kg/h)</td>
<td>157.18</td>
<td>146.23</td>
<td>138.15</td>
<td>130.12</td>
<td>127.21</td>
<td>111.21</td>
<td>100.12</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>13.5</td>
<td>15.6</td>
<td>15.9</td>
<td>16.2</td>
<td>16.8</td>
<td>17.5</td>
<td>18.9</td>
</tr>
<tr>
<td>TA (%)</td>
<td>0.494</td>
<td>0.745</td>
<td>0.701</td>
<td>0.783</td>
<td>0.796</td>
<td>0.754</td>
<td>0.652</td>
</tr>
<tr>
<td>TSS/TA</td>
<td>27.33</td>
<td>20.94</td>
<td>22.68</td>
<td>20.69</td>
<td>21.11</td>
<td>23.21</td>
<td>28.99</td>
</tr>
<tr>
<td>Glucid (%)</td>
<td>5.84</td>
<td>6.37</td>
<td>7.23</td>
<td>7.91</td>
<td>8.20</td>
<td>8.73</td>
<td>8.51</td>
</tr>
<tr>
<td>Ratio of flesh/fruit (%)</td>
<td>47.14</td>
<td>50.12</td>
<td>53.71</td>
<td>59.90</td>
<td>64.21</td>
<td>71.10</td>
<td>72.67</td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td>12.8</td>
<td>11.3</td>
<td>8.8</td>
<td>6.4</td>
<td>4.0</td>
<td>3.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Skin hardness (%kg/cm²)</td>
<td>4.525</td>
<td>4.831</td>
<td>4.957</td>
<td>5.235</td>
<td>5.278</td>
<td>6.089</td>
<td>5.585</td>
</tr>
<tr>
<td>Sensory Test</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>7.5</td>
<td>8.5</td>
<td>9.0</td>
</tr>
</tbody>
</table>

CONCLUSION
This study indicates the harvesting indexes including harvesting time (from after flowering to harvesting), respiration rate, sensory characteristics (appearance, colour, odour, taste, texture,...), physio-chemical properties (TSS, moisture content, glucid, lipid, organic acid, Vitamin C...) and other specifications (hardness, skin thickness, links of fruit & peduncle...) of six different kinds of fruit, such as: ‘Lo Ren” star-apple, “Sugar” mandarin, “Yellow Pulp & Dwindled Seed” durian, “Sanh” orange, “Green Skin” pomelo and mangosteen. The results also indicated the proper harvesting time for each of six fruits according to its consuming purposes: fresh consumption, processing and storage.
REFERENCES


ANTIBIOTIC RESISTANCE OF ENTERIC BACTERIA ISOLATED FROM RAW FOOD IN VIETNAM

Thi Thu Hao Van, George Moutafis, Taghrild Istivan, Linh Thuoc Tran and Peter J Coloe

ABSTRACT

A study to examine the contamination rate and antibiotic resistance of enteric bacteria in 180 raw food samples in Vietnam was conducted. Salmonella spp. was found in 60.8% of meat and 18.0% of shellfish samples and Escherichia coli was present in more than 90% of the samples. All isolates were screened for antibiotic resistance against 15 antibiotics. Multi-resistance, defined as resistance to at least three different classes of antibiotics, was detected in isolates from all food sources. Integrons were found in 57% of E. coli and 13% of Salmonella spp. isolates. Nucleotide sequencing results revealed that the integrons harboured different gene cassettes, including genes responsible for resistance to aminoglycosides, ampicillin, trimethoprim, and chloramphenicol. Plasmids were detected in all 23 antibiotic resistant Salmonella spp. and in 33 E. coli isolates with 35% of the former and 76% of the latter contained plasmids larger than 95 kb. Antibiotic resistance phenotypes were found to be transferable among the Salmonella spp. and E. coli isolates. All multi-resistant Salmonella spp. isolates were examined for the presence of Salmonella genomic island 1 (SGI1) antibiotic resistance gene cluster and one isolate from chicken (serovar Albany) contained a variant of this island. Our results show that antibiotic resistance in enteric bacteria isolates from Vietnamese raw food samples is significant.
STUDY ON APPROPRIATE PACKING AND OPTIMUM STORAGETEMPERATURE FOR SOME SPECIFIC FRUITS

Nguyen Vu Hong Ha, Le Minh Hung
Southern Sub-Institute of Agricultural Engineering and Post Harvest Technology,
Hochiminh city, Vietnam. Email: hong_ha2001vn@yahoo.com

ABSTRACT

Inheritance of the results obtained from the study on determining harvesting indexes for six specific kinds of fruit in Tien Giang, Ben Tre, Dong Thap, Vinh Long, Binh Duong and Ho Chi Minh city, the study on identifying appropriate packing and optimal storage temperature for these fruits has been carried out to prolong their shelf-life and increase their quality in storage in order to reduce fruit production costs for farmers and give a reasonable prices to both traders and consumers. Packing and coating fruits using various types of plastics and of membranes were tested after each week of storage to determine their capabilities to extend fruit shelf-life. The results indicated that the storage duration of “Lo Ren” star-apple was 28-30 days when packed in PA bags and stored at 10 ± 1°C while that of “Sanh” orange was 70-75 days when packed in PE bags and stored at 8 ± 1°C; that of “Green Skin” pomelo was 55-60 days, packed in PE bags and stored at 10 ± 1°C; that of “Lai Thieu” mangosteen was 42-45 days, packed in PE bags and stored at 10 ± 1°C; that of “Sugar” mandarin was 11-12 weeks when packed in OTR 2000 bags and stored at 6 ± 1°C, and that of “Yellow Pulp & Dwindled Seed” durian was 28-30 days, packed in paraffin wax & cartons and stored at 12 ± 1°C.

Keywords: packing, plastic, membrane, shelf-life, star-apple

INTRODUCTION

The fruit industry in Vietnam has a great potential and plays an important role in agricultural production. In 2003, Vietnam exported US$43 million of high value fruit to high-income countries and imported US$14 million of fruit and vegetables (Ha, N.V.H, 2005). Located in the tropical and temperate zone, southern provinces of Vietnam are more likely to favourably develop agricultural production and provide various types of high quality fruits such as citrus, mangosteen, durian and star apple. However, between 30 to 40 per cent of fruit produced in Viet Nam is believed to be lost and does not reach the market. This can be due to its improper post-harvest handling where farmer's fruits orchards are often spread out over a wide area and damage may occur when fruits are transported from the orchard to the packing station or collection point in which bamboo baskets are still used as the commonly packaging technique to prevent mechanical damage. Moreover, physiological changes in storage cause many losses in quality and quantity of fruits (Thoa, N.V., 1982). Many techniques have been studied in attempt to solve these problems and one of them is using plastic packing and membranes coating to preserve and extend the shelf-life of fruits (Hoan, N.C. et al, 2001). According to Wills et al. (1989), regarding preservation purposes, these packing or coating must comply with the requirements in the respects of sufficient mechanical strength, free of chemical substances that could become toxic to man, the permeability of plastic films to respiratory gases, light or transparency...

The aim of this research was to study on suitable plastic packing and membrane coating for extending the shelf-life period of six particular fresh fruits in southern provinces of Vietnam.
MATERIALS AND METHODS

Materials

Six particular fruits of Southern Vietnam including “Sugar” mandarin, “Sanh” orange, “Green Skin” pomelo, “Lai Thieu” mangosteen, “Lo Ren” star-apple and “Yellow Pulp & Dwindled Seed” durian were collected and selected for the study.

Some plastics such as PE (poly ethylene), OTR 2000 (Oxygen transmission rate of 2000ml oxy/h/m²), OTR 4000 (Oxygen transmission rate of 4000ml oxy/h/m²), PA (Polyamid), fibreboard carton boxes and biopolymer membrane including chitosan and paraffin wax were selected for the experiments.

PE with punched holes can be used to improve gas permeability (the diameter of punched hole was 0.1mm).

Methods

Packing and coating fruits using various types of plastics and of membranes were compared with the controls (no packing or coating) and tested after each week of storage to determine their capabilities to extend fruit shelf-life. Testing periods varied from 5 to 12 weeks according to each fruit type.

Changes in weight loss, total soluble substance (0Bx), organic acid, hardness (kg/cm²), Vitamin C content (%mg) and CO2 concentration (ppm) inside the bags were measured and recorded to determine the capabilities of either packing or coating in extending fruit shelf-life.

- **Weight losses of fruit** were determined by scale (g or kg).
- **Fruit hardness (kg/cm²)** was measured by penetrometer.
- **TSS (Brix)** was measured by digital refractometer.
- **Total acidity (organic acidity)** was determined by measurement of titration with NaOH 0,1N using phenolphthalein 1% as indicator.
- **Vitamin C content** was determined by measurement of titration with 2,6 diclophenol - indophenol 0,001%.
- **Glucid content (%):** Total glucid indicated as glucose or inverted sugar in 100g was measured as following formula:
  \[
  X = \frac{(G1 \times 100) \times \text{Dilution degree}}{(G \times 1000)}
  \]
  
  G1: weight of inverted sugar or glucose (mg) equivalent to KMnO₄ 0,1N.
  G: Initial weight of sample (g)
  1000: convert from mg to g.
  Dilution degree varies according to fruit type

CO₂ concentration was measured by a hand-held Carbon Dioxide meter (Gas PCO2/10).

Three samples of each fruit type were selected for experiments (number of replications: 3).

Six fruits at different maturity degrees were tested under the optimal conditions concerning temperature and humidity which had been recognised by the previous study on “determining harvesting indexes of some specific fruits in order to improve their quality in preservation and processing” conducted by SIAEP (Ha, N.V.H., 2005).

Among six fruit types studied, star-apple was selected to be presented in this paper. The experiment design, results and conclusion of study on “Lo Ren” star-apple was introduced.
while only the conclusion of studies on “Sugar” mandarin, “Sanh” orange, “Green Skin” pomelo, “Lai Thieu” mangosteen and “Yellow Pulp & Dwindled Seed” durian were presented in this paper.

Experiment methods for “Lo Ren” star-apple:

- PA.
- PE0 + Chitosan 1% (PE0 : PE without holes punched).
- PA + Chitosan 1%.
- Control (neither packing nor coating)

Experiments for star-apple were carried out at the storage temperature of 10±1°C and the humidity of 85±90%.

RESULTS AND DISCUSSION

**Weight loss (%):**

*Table 1. Plastic packing affecting weight losses of “Lo Ren” star-apple*

<table>
<thead>
<tr>
<th>Plastic packing</th>
<th>Duration (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>1.77</td>
</tr>
<tr>
<td>PE0 + Chitosan 1%</td>
<td>0</td>
</tr>
<tr>
<td>PA + Chitosan 1%</td>
<td>1.32</td>
</tr>
<tr>
<td>Control (9 days)</td>
<td>8.81 (*)</td>
</tr>
</tbody>
</table>

Ghi chú: (*): Skin starts to shrivel

**Change in total soluble solids-TSS (%Bx):**

*Table 2. Plastic packing affecting the total soluble solids of “Lo Ren” star-apple*

<table>
<thead>
<tr>
<th>Plastic packing</th>
<th>Duration (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>14.5</td>
</tr>
<tr>
<td>PE0 + Chitosan 1%</td>
<td>14.3</td>
</tr>
<tr>
<td>PA + Chitosan 1%</td>
<td>14.2</td>
</tr>
<tr>
<td>Control (9 days)</td>
<td>11.7</td>
</tr>
</tbody>
</table>

**Change in hardness (kg/cm²):**

*Table 3. Plastic packing affecting hardness of “Lo Ren” star-apple*

<table>
<thead>
<tr>
<th>Plastic packing</th>
<th>Duration (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>1.68</td>
</tr>
<tr>
<td>PE0 + Chitosan 1%</td>
<td>1.40</td>
</tr>
<tr>
<td>PA + Chitosan 1%</td>
<td>1.57</td>
</tr>
<tr>
<td>Control (9 days)</td>
<td>1.02</td>
</tr>
</tbody>
</table>
### Change in Glucid content (%):

**Table 4: Plastic packaging affecting glucid content of star-apple**

<table>
<thead>
<tr>
<th>Plastic packing</th>
<th>Duration (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>9.10</td>
</tr>
<tr>
<td>PE0 + Chitosan 1%</td>
<td>9.24</td>
</tr>
<tr>
<td>PA + Chitosan 1%</td>
<td>9.03</td>
</tr>
<tr>
<td>Control (9 days)</td>
<td>6.54</td>
</tr>
</tbody>
</table>

### Change in CO2 concentration (ppm):

**Table 5: Plastic packaging affecting CO2 concentration of star-apple**

<table>
<thead>
<tr>
<th>Plastic packing</th>
<th>Duration (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>11.98</td>
</tr>
<tr>
<td>PE0 + Chitosan 1%</td>
<td>15.20</td>
</tr>
<tr>
<td>PA + Chitosan 1%</td>
<td>11.20</td>
</tr>
</tbody>
</table>

After 9-day storage: the star-apples from the control started to deteriorate and their skin turned brown, shrivelled and soft.

After 4 weeks: the star-apples from the experiment with PA showed better quality and longer shelf-life than those from either experiment with PE0+ Chitosan 1% or that with PA + Chitosan 1%.

The experiment with PE0+ Chitosan 1%: after 3 weeks the star-apple’s skin was getting soft and the fruits occurred big bruises on their surfaces due to physiological disorders and sogginess of fruits resulting from tightly packing bags and acids accumulated by dissolving Chitosan.

The experiment with PA+ Chitosan 1%: after 3 weeks the weight loss was higher than that of the experiment with PA perhaps due to the acid content accumulated from Chitosan dissolution speeding up the water evaporation and resulting in physiological disorders of star-apples (Mitra, 1997).

The experiment with PA: after 4 weeks star apples maintained good quality in term of appearance and nutrional values. PA prevented physiological disorders of fruits whilst in storage. After 5-week storage, star-apples started to deteriorate.

**CONCLUSION**

PA bag was the optimal packing for “Lo ren” star apple and able to extend its shelflife when stored at 10 ± 1°C up to 4 weeks equivalent to 28-30 days.

Other results also indicated that the storage duration of “Sugar” mandarin was 11-12 weeks when packed in OTR 2000 bags and stored at 6 ± 1°C, while that of “Sanh” orange was 70-75 days when packed in PE bags and stored at 8 ± 1°C; that of “Green Skin” pomelo was 55-60 days, packed in PE bags and stored at 10 ± 1°C; that of “Lai Thieu” mangosteen was 42-45 days, packed in PE bags and stored at 10 ± 1°C; and that of
“Yellow Pulp & Dwindled Seed” durian was 28-30 days, packed in paraffin wax & cartons and stored at 12 ± 1°C.

REFERENCES


Session 2

Novel Food Processing and Preservation Technology
INTRODUCTION

High pressure processing (HPP) of foods is gaining popularity in the food industry in part because of its ability to inactivate vegetative cells of microorganisms and enzymes near room temperature, resulting in the almost complete retention of nutritional and sensory characteristics of fresh food without sacrificing shelf life (Tauscher 1995; Knorr and Heinz 2001). Typically, pressures up to 700 MPa and treatment times from a few seconds to several minutes are used to inactivate bacterial cells. As opposed to conventional thermal processing, hydrostatic pressurization allows “instant” pressure transmission into fluids and semisolids within the pressure vessel, thereby achieving short processing times. HPP has the potential to deliver preservative-free products that are safe and have good shelf-life, particularly for low pH products. This technology has already successfully been applied by more than 60 companies worldwide for the production of fresh-like foods (e.g. avocado products and juices, ready-to-eat meats and meals) and for the improvement of shucking efficiency and seafood safety.

PRINCIPLES OF HIGH PRESSURE PROCESSING OF FOODS

The basis of the efficacy of high-hydrostatic-pressure processing is Le Chatelier’s principle, in that reactions or phase transitions associated with a decrease in volume during pressurisation are favoured, whilst those accompanied with a volume increase are inhibited. The degree of contraction is governed by the compressibility $\beta$, which is dependent on the intermolecular forces acting within the substance. The volume reduction of water and water-based foods at 600 MPa and room temperature is approximately 15%.

All compressible substances change temperature during physical compression and this is an unavoidable thermodynamic effect. Of the major food components, water has the lowest compression heating values, while fats and oils have the highest. For example, at pressures normally encountered during HPP (400–800 MPa), under adiabatic conditions near room temperature, water typically changes 3°C for every 100 MPa pressure change. Since water is the main ingredient in most foods, adiabatic temperature changes exhibited by most foods are very similar to that of water. However, fats and oils show significant higher compression heating values of 6 to 9°C per 100 MPa (Rasanayagam et al. 2003).

EFFECTS OF HIGH PRESSURE ON FOOD COMPONENTS

The primary structure of low molecular weight molecules such as vitamins, peptides, lipids, and saccharides is rarely affected by high pressure because of the very low compressibility of covalent bonds at pressures < 2000 MPa (Gross and Jaenicke 1994; Cheftel and Culioli 1997; Van den Broeck et al. 1998; Oey et al. 2006). On the other hand, certain macromolecules, such as proteins and starches, can change their native structure during HPP, in a manner analogous to thermal treatments (Heremans 1982; Cheftel 1992). For example, it has been reported that starch granules and/or protein solutions can form a gel due to pressurisation (Stolt et al. 2000).

With particular regard to proteins, primary structure is not affected by pressure. However, pressure influences the quaternary structure of the protein through hydrophobic-interactions, the tertiary structure through reversible unfolding, and the secondary
structure through irreversible unfolding. At room temperature, pressure induced changes in proteins and enzymes are generally reversible in the pressure range 100–400 MPa and irreversible for pressures above 400 MPa. Pressure also favours the dissociation of oligomeric proteins, as well as unfolding of protein chains (Tauscher 1995).

EFFECTS OF HIGH PRESSURE ON MICROORGANISMS

The main application of HPP in the food industry is for the extension of shelf-life or for the elimination of microbial pathogens. The viability of vegetative microorganisms is affected by inducing structural changes at the cell membrane or by the inactivation of enzyme systems which are responsible for the control of the metabolic actions (Knorr and Heinz 2001). At pressures higher than 400 MPa a significant inactivation of vegetative bacteria, yeasts and viruses has been observed at ambient temperatures within minutes (Farkas and Hoover 2001; Isbarn et al. 2007; Lori et al. 2007). By increasing the pressure to 800 MPa (at present the technical pressure limit for the high-end industrial high pressure equipment) most inactivation reactions are strongly accelerated.

Bacterial endospores, as compared to vegetative cells, display a considerably higher resistance to high pressure. Spores of *Clostridium botulinum* and *Bacillus* species are the key bacteria for the safety or the spoilage of low acid (heat treated) preserved goods. These spores have shown remarkable tolerance to pressures above 1000 MPa at room temperature (Margosch et al. 2004; Margosch et al. 2006). However, many other bacterial endospores, which are relevant to food are inactivated at pressures 600 MPa or greater in combination with initial temperature above 60°C (Heinz and Knorr 2002). Often the required inactivation temperature and/or time is lowered by combination with pressure.

EFFECTS OF HIGH PRESSURE ON FOOD STRUCTURE

Apart from its food preservation capabilities, HPP has also shown good potential to manipulate the functionality of food ingredients such as fats, proteins and carbohydrates. Important food quality attributes such as taste, texture and appearance are closely linked to the material and microstructure properties of a food, which are in turn derived from a combination of formulation and process design. There are now increasing examples of processes which make use of high pressure technology for the modification of food properties or functionality. In Japan, rice is pressurised at 400 MPa and 60°C to enhance the flavour and to reduce the allergenic potential of this ready-to-eat rice product. Modified dairy and egg-based ingredients of varied functionality, and tenderised meats are further examples of the benefits that can be achieved in texture modification (Jimenez-Colmenero 2002). Another application is in oysters that are treated at about 300 MPa for preshucking to increase consumer convenience.

CONCLUSIONS

Pressure is a processing variable that can drastically influence food functional properties. Inactivation of microorganisms and enzymes at low or moderate temperatures whilst retaining sensory and nutritional properties shows that high pressure technology has the potential to be used in the development of a whole new generation of value added foods. The use of HPP under controlled conditions also allows for greater ingredient flexibility in designing and constructing food matrices and provides a tool to enhance quality, reduce cost and/or achieve novel properties. Like any other food preservation processes, HPP is product specific, making shelf life extension of food stuff dependent on pH, food composition, intrinsic enzymes, and on the actual bacterial species/strains present.

HPP is not likely to replace all traditional processing methods, but it may complement such methods or find niche applications. In addition, novel physico-chemical and sensory properties obtained from this technology offer exciting opportunities for industry. High
capital costs may limit its application initially but this will be offset by lower operating costs since the energy required for HPP is less than the energies used in thermal processing.

REFERENCES


EFFECT OF MILD-HEAT AND HIGH-PRESSURE PROCESSING ON BANANA PECTIN METHYLESTERASE: A KINETIC STUDY

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² Department of Food Technology, College of Agriculture, Cantho University, Campus 2, 3/2 Str., Cantho city, Vietnam

ABSTRACT

Pectin methylesterase (PME) was extracted from bananas and purified by affinity chromatography. The thermal-high pressure inactivation (at moderate temperature, 30 to 76 °C, in combination with high pressure, 0.1 to 900 MPa) of PME was investigated in a model system at pH 7.0. Under these conditions, the stable fraction was not inactivated and isobaric-isothermal inactivation followed a fractional-conversion model. At lower pressure (≤ 300 to 400 MPa) and higher temperature (≥ 64 °C), an antagonistic effect of pressure and heat was observed. Third-degree polynomial models (derived from the thermodynamic model) were successfully used to describe the heat pressure dependence of the inactivation rate constants.

Keywords: pectin methylesterase, high pressure, inactivation kinetics, thermodynamic model

INTRODUCTION

Among nonthermal processing technologies, high-pressure processing is being applied on an industrial scale for some food products marketed in Japan, the United States, and some European countries (Heremans, 2002). High-pressure processing, in contrast to high-temperature processing, shows a higher specificity towards maintaining the fresh quality of foods because it slightly affects covalent bonds (in the pressure range used). Hence, high-pressure processing at room temperature has very little detrimental effect on food quality attributes such as vitamins, pigments, and flavors (Cheftel, 1991; Heremans, 1993; Knorr, 1993; Eshtiaghi et al., 1994; Kimura et al., 1994) and can supply consumers with fresher and higher-quality safe food products.

The enzyme PME (EC 3.1.1.11), which has been found in plants as well as in pathogenic fungi and bacteria, catalyzes the hydrolysis of the methyl ester groups from pectin and leads to the formation of a calcium pectate gel (Walkinshaw and Arnott, 1981; Powell et al., 1982; Ben-Shalom et al., 1985; Alonso et al., 1995). Consequently, its activation, on the one hand, causes cloud loss of juices and nectars (Krop and Pilník, 1974; Rothschild and Karsenty, 1974; Rombouts et al., 1992; Laratta et al., 1995), on the other hand, (i) enhances the texture of fruit and vegetable-based products (Alonso et al., 1995; Fuchigami et al., 1995; Alvarez et al., 1999), (ii) effectively increases the extracting yield of juices by conventional methods (Anastasakis et al., 1987), and (iii) promotes water removal from the tissues on drying (Manabe, 1982).

Plant PME have been isolated, purified, and studied in terms of pressure-thermal processing stability. In this context, commercial tomato PME was found to be activated under lower pressure (< 300 MPa) treatment at mild temperature (60 to 65°C) (Van den Broeck et al., 2000a). Similarly, purified strawberry PME showed extreme pressure stability toward combined pressure-temperature treatment up to 1000 MPa at 10 °C (Ly
Nguyen et al., 2002a). For orange PME, a pressure-temperature kinetic diagram for inactivation was published showing an antagonistic effect of pressure and temperature at pressures below 300 MPa and temperatures above 60 °C (Van den Broeck et al., 2000b). Thus, high-pressure treatment can activate or inactivate plant PME’s, depending on the pressure temperature level applied. Both effects (activation and inactivation) on PME can be beneficial in the processing of fruit and vegetable-based products. Pressure-temperature processing stability data for plant PME’s, therefore, are of interest to the food industry.

In the present work, a detailed kinetic study was performed using banana PME. The processing stability of purified banana PME in a model system (i.e., in 20 mM Tris-HCl buffer, pH 7.0) was investigated using combined heat-pressure treatments. This fruit was selected because high-pressure processing might be of interest for the processing of banana juice and nectar.

MATERIALS AND METHODS

Materials
A stock of 15 kg of bananas (Bonita banana: cv Cavendish, Ecuador) was purchased from a supermarket. Apple pectin (DE 70-75%) was obtained from Fluka Chemical Co. (Buchs, Switzerland). All other chemicals were of analytical grade. PME was extracted from bananas and purified by affinity chromatography on a CNBr-Sepharose 4B-PME inhibitor column (Ly Nguyen et al., 2002b). Purified banana PME obtained was desalted and dissolved in 20 mM Tris-HCl buffer (pH 7.0) (specific activity is 480 units/mg protein), quickly frozen using liquid nitrogen, and stored at –80 °C for further use.

PME assay
PME activity was measured by continuous recording of the titration of carboxyl groups released from a pectin solution using an automatic pH-stat (Metrohm, Herisau, Switzerland) and 0.01 N NaOH. Routine assays were performed with a 3.5-mg mL⁻¹ apple pectin solution (30 mL) containing 0.117 M NaCl (pH 7.0) at 22.5 °C. The activity unit (U) of PME is defined as the amount of enzyme required to release 1 µmol of carboxyl groups per minute, under the aforementioned assay conditions (Hou et al., 1997).

Heat inactivation of purified banana PME
Isothermal treatments were performed in a temperature-controlled water bath using 200-µL capillaries (Blaubrand, Wertheim, Germany) to enclose the enzyme solution. After treatment, the samples were immediately cooled in ice water. Residual activities of PME were measured within 60 min of storage in ice water. Previous experiments showed the absence of reactivation of the enzyme during this time period.

Combined heat-pressure inactivation of purified banana PME
All combined heat-pressure experiments were conducted in a multi-vessel, high-pressure apparatus (eight vessels of 8 mL) (Resato, Roden, The Netherlands), which allows pressurization up to 1000 MPa in combination with temperatures ranging from –20 to 100 °C. The pressure medium is a glycol-oil mixture (TR 15, Resato). Enzyme samples in 0.3-mL flexible micro tubes (Elkay, Leuven, Belgium) were enclosed in the pressure vessels, already equilibrated at the inactivation temperature. Pressure was built up slowly using a standard pressurization rate of about 100 MPa/min to minimize the temperature rise due to adiabatic heating (Weemaes et al., 1997; Ludikhuyze et al., 1998). After pressure build-up, an equilibration period of 2 min to allow temperature of pressure medium to evolve to its preset value (input value) was taken into account (Van den Broeck et al., 2000b). After 2 min equilibration, one pressure vessel was decompressed and the activity of the corresponding enzyme sample was considered as the blank (Ao). The other seven vessels,
each containing one enzyme sample, were then decompressed after certain periods as a function of time. After pressure release, samples were immediately cooled in ice water and the residual PME activity was measured within 60-min storage time in ice water. The experiments were performed at combined pressures and temperatures ranging from 100 to 900 MPa and from 30 to 76 °C.

**Kinetic data analysis**

As previously published by Ly Nguyen and co-workers (2002b), the heat-pressure inactivation of purified banana PME followed a fractional-conversion model. This model applies when the enzyme sample contains a stable fraction that is not affected under the processing condition studied.

\[
A = A_o + (A_o - A_o) \exp(-kt) \quad (1)
\]

where \( A_o \) and \( A \) are respectively the initial and the remaining activities at time \( t \) (min); \( A_o \) is the remaining activity after prolonged treatment (mL of 0.01 N NaOH/min); \( k \) is the inactivation rate constant (min\(^{-1}\)).

Many mathematical models have been formulated that allow the description of temperature-pressure dependence of the inactivation rate constant over a broad range of pressures and temperatures. The most useful thermodynamic-based kinetic model governing the behavior of a system during pressure and temperature change (eq 2) was used as a general equation to describe the heat-pressure inactivation of purified banana PME (Hawley, 1971; Morild, 1981; Weemaes et al., 1998). This model has been successfully applied as a generic model for a number of enzyme inactivation data and can currently be regarded as the most generic model in this field (Indrawati, 2002).

\[
\ln(k) = \ln(k_o) - \frac{\Delta V_o}{R_o T} (P - P_o) + \frac{\Delta S_o}{R_o T} (T - T_o) - \frac{\Delta \kappa}{2 R_o T} (P - P_o)^2 + \frac{\Delta C_P}{R_o T} \left\{ T \left[ \ln \left( \frac{T}{T_o} \right) - 1 \right] + T_o \right\} - \frac{2 \Delta \zeta}{R_o T} (P - P_o)(T - T_o) \quad (2)
\]

where \( P \) is pressure (MPa); \( T \) is absolute temperature (K); \( P_o \) and \( T_o \) are reference pressure (MPa) and absolute temperature (K), respectively; \( \Delta V_o \), \( \Delta S_o \) are volume change (cm\(^3\) mol\(^{-1}\)), and entropy change (J mol\(^{-1}\) K\(^{-1}\)) between native and denatured states, respectively; \( \Delta \kappa \) is compressibility factor (cm\(^6\) J\(^{-1}\) mol\(^{-1}\)); \( \Delta C_P \) is heat capacity (J mol\(^{-1}\) K\(^{-1}\)); \( \Delta \zeta \) is thermal expansibility factor (cm\(^3\) mol\(^{-1}\) K\(^{-1}\)); \( k \) is inactivation rate constant (min\(^{-1}\)); \( k_o \) is inactivation rate constant at \( P_o \) and \( T_o \) (min\(^{-1}\)); \( R_o \) is universal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)).

Recently, Smeller (2002) suggested a possible modification, in the vicinity of the reference point, one can use the following second order approximation:

\[
T \left[ \ln \left( \frac{T}{T_o} \right) - 1 \right] + T_o = \frac{(T - T_o)^2}{2 T_o} \quad (3)
\]

This approach results in a second-degree polynomial (elliptic) equation:

\[
\ln(k) = \ln(k_o) - \frac{\Delta V_o}{R_o T} (P - P_o) + \frac{\Delta S_o}{R_o T} (T - T_o) - \frac{\Delta \kappa}{2 R_o T} (P - P_o)^2 + \frac{\Delta C_P}{R_o T} \left( T - T_o \right)^2 \quad (4)
\]
In cases $\Delta \kappa$, $\Delta C_p$, and $\Delta \zeta$ are temperature and/or pressure dependent (Yamaguchi et al., 1995; Smeller and Heremans, 1997), an extended analysis of the free-energy change $\Delta G(T, P)$ (in their cases) or $\ln(k)(T, P)$ (in the present case) is necessary, where higher-order terms are also involved.

$$
\ln(k) = \ln(k_0) - \frac{\Delta V^*}{R_T T}(P - P_o) + \frac{\Delta S^*}{R_T T}(T - T_o) - \frac{\Delta \kappa^*}{2R_T T}(P - P_o)^2 + \frac{\Delta C_p^*}{R_T T}(T - T_o)^2
$$

$$- \frac{2\Delta \zeta^*}{R_T T}(P - P_o)(T - T_o) + \text{ higher-order terms} \quad (5)
$$

In general, there are 4 third-degree terms (Ly Nguyen et al., 2003):

$$
\frac{\Delta \kappa^*}{2RT}(P - P_o)^3; \quad \frac{\Delta C_p^*}{2RT T_o}(T - T_o)^3; \quad \frac{2\Delta \zeta^*}{RT}(P - P_o)^2 (T - T_o); \quad \text{and}
$$

$$
\frac{2\Delta \zeta^*}{RT}(P - P_o)(T - T_o)^2
$$

Note that the subscript ‘2’ refers to the coefficients of the higher-order terms; however, in case of pressure-temperature inactivation of banana PME the term $\frac{\Delta C_p^*}{2RT T_o}(T - T_o)^3$ is redundant as indicated by the large standard error (100%). As a consequence, this term was omitted and a reduced version of eq 5 was used (i.e. eq 6).

$$
\ln(k) = \ln(k_0) - \frac{\Delta V^*}{R_T T}(P - P_o) + \frac{\Delta S^*}{R_T T}(T - T_o) - \frac{\Delta \kappa^*}{2R_T T}(P - P_o)^2 + \frac{\Delta C_p^*}{R_T T}(T - T_o)^2
$$

$$- \frac{2\Delta \zeta^*}{R_T T}(P - P_o)(T - T_o) + \frac{\Delta \kappa^*}{2RT}(P - P_o)^3 + \frac{2\Delta \zeta^*}{RT}(P - P_o)^2 (T - T_o)
$$

$$+ \frac{2\Delta \zeta^*}{RT}(P - P_o)(T - T_o)^2 \quad (6)
$$

Equation 6 can be rewritten as an empirical polynomial equation as its parameters have no longer a physical meaning:

$$
\Rightarrow \ln(k) = A + B(P - P_o) + C(T - T_o) + D(P - P_o)^2 + E(T - T_o)^2 + F(P - P_o)(T - T_o)
$$

$$+ G(P - P_o)^3 + I(P - P_o)^2 (T - T_o) + J(P - P_o)(T - T_o)^2 \quad (7)
$$

where $A, B, C, D, E, F, G, I,$ and $J$ are unknown parameters.

As measures for the quality of model fitting, the corrected $r^2$ and the model standard deviation ($SD$) were calculated using eq 8 and eq 9, respectively.

$$
corrected r^2 = 1 - \frac{SSQ_{\text{regression}}}{SSQ_{\text{total}}} \quad (8)
$$

$$
SD = \sqrt{\frac{SSQ_{\text{residual}}}{(m - j)}} \quad (9)
$$
RESULTS AND DISCUSSION

Heat-pressure inactivation kinetics of purified banana PME. Heat-pressure inactivation experiments of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) were conducted for different combinations of moderate temperature (30 to 76 °C) and pressure (0.1 to 900 MPa). The isothermal-isobaric inactivation of purified banana PME followed a fractional-conversion model (eq 1). There is an antagonistic effect of pressure and temperature in the ‘low’ pressure ($P \leq 300$ to $400$ MPa) high temperature domain ($\geq 64°C$, in the present study). In this range, a pressure increase resulted in a decrease of the observed inactivation rate constant. An antagonistic effect of pressure and temperature is frequently encountered for enzyme inactivation/protein denaturation. This effect is mostly limited to pressures below 300 MPa (Balny and Masson, 1993; Heremans, 1993; Mozhaev et al., 1996; Weemaes et al., 1998; Van den Broeck et al., 2000b; Indrawati et al., 2001). Pressure stabilization of enzymes/proteins against thermal inactivation/denaturation might be due to counteracting effects of pressure and temperature on the formation or disruption of intramolecular interactions and/or to their opposing effects on interactions between enzyme/protein and solvent (water).

Discussing the interaction of enzyme/protein functional groups and solvent (water), Gross and Jaenicke (1994), Mozhaev and co-workers (1996), and Barbosa-Cánovas and coworkers (1997) stated that in the initial step of thermal inactivation, a protein loses a number of essential water molecules, and this loss may give rise to structural rearrangements. High pressure may hamper this process owing to its favorable effect on hydration of both charged and nonpolar groups (Heremans, 1982; Gross and Jaenicke, 1994).

Opposing effects of pressure and temperature with respect to hydrophobic interactions and hydrogen bonds have furthermore been put forward as possible explanations for pressure stabilization of enzymes/proteins against thermal inactivation/denaturation. Endothermic hydrophobic interactions are known to be enhanced at elevated temperatures, being maximal at about 60 to 70 °C and thereafter decreasing because of a gradual breakdown of the water structure (Damodaran, 1996). Pressure, on the other hand, greatly weakens hydrophobic interactions (Cheftel, 1991; Balny and Masson, 1993). As to hydrogen bridges, it is generally accepted that these interactions are destabilized by elevated temperature. Pressure, on the other hand, often stabilizes hydrogen bridges (Cheftel, 1991; Heremans, 1993; Damodaran, 1996).

Modeling of combined heat-pressure dependence of inactivation rate constants. Fitting eq 4, eq 6, and eq 7 on the experimental data, the model parameters were estimated using nonlinear regression analysis (Proc NLIN, SAS).

For the three model versions, no tendency was found by plotting residuals (differences between experimental and predicted $k$-values, respectively) as a function of temperature, pressure, experimental $k$-value, and predicted $k$-value (data not shown). In addition, parity plots of the natural logarithm of the predicted $k$-values based on eq 4, eq 6, and eq 7 versus the natural logarithm of the experimental $k$-values, respectively, were established (Figure 1). The deviation from the bisector can be considered as an indicator for the inaccuracy of the models. The less the experimental and predicted $k$-values mutually differ, the more successful the model is. Good agreements between the natural logarithm of the predicted $k$-values and that of the experimental $k$-values were observed for the three model versions. It is obvious that the third-degree models better fit the experimental data ($R^2=0.9903$ (eq 6) and 0.9906 (eq 7)) as compared to the ‘classical’ model ($R^2 = 0.8797$ (eq 4)). Graphically,
the third-degree models show better fitting in the areas of low temperature – high pressure and high temperature – low pressure (Figure 2). A similar conclusion was drawn to the heat-pressure inactivation of purified carrot PME (Ly Nguyen et al., 2003). In addition, a comparison of correlation coefficients and residuals based on eq 6 and eq 7 allowed us to conclude that the third-degree polynomial model with unknown parameters (eq 7) can also be used to adequately describe the heat-pressure dependence of inactivation rate constants of banana PME. However, since there is no big difference between quality of the third degree thermodynamic, and the third degree polynomial fittings, the thermodynamic model should be favored, because this is the theoretically correct one. In case of the polynomial model, the 1/RT factor is built into the constants of the polynomial and therefore the change of the temperature is not taken into account exactly.

Figure 1. Correlation between the natural logarithm of the experimental $k$-values of the isobaric-isothermal inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) and the natural logarithm of the predicted $k$-values according to: (A) the ‘classical’ thermodynamic model (eq 4); (B) the third-degree thermodynamic model (eq 6); and (C) the third-degree polynomial model (eq 7).

Figure 2. 3D plots for heat-pressure inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) based on (A) the ‘classical’ thermodynamic model (eq 4); (B) the third-degree thermodynamic model (eq 6); and (C) the third-degree polynomial model (eq 7). (O) are raw data points.

By inserting all estimated model parameters into eq 4, eq 6, and eq 7, respectively, pressure-temperature combinations resulting in specific pre-set inactivation rate constants for purified banana PME were simulated and depicted in iso-rate contour plots (Figure 3). Smeller and Heremans in 1997 discussed that as one applies the thermodynamic model with only 1$^{st}$ and 2$^{nd}$ order terms the shape of contour plot will be elliptic or hyperbolic. To leave out the higher order terms is equivalent to the assumption that $\Delta k$, $\Delta C_P$, and $\Delta \zeta$ are independent of temperature and pressure. If any of these shows temperature or
pressure dependence higher-order terms appear not to be negligible. In cases higher-order terms become important and are included in the model, the contour plot will be a distorted ellipse.

**Figure 3.** Heat-pressure iso-rate contour plots of 95% inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) for a total process time of 30 min (k = 0.1 min⁻¹) based on (A) the ‘classical’ thermodynamic model (eq 4); (B) the third-degree thermodynamic model (eq 6); (C) the third-degree polynomial model (eq 7); and (D) of raw data.

**REFERENCES**


ABSTRACT

Grain hydration is a time consuming process, and the objective of this work was to determine if it is possible to enhance hydration by the use of high power ultrasound (HPU). When paddy rice was hydrated at 50°C or 60°C, it was shown that HPU increased the rate of hydration, with a larger effect at 50°C than 60°C. RVA analysis of starch extracted from conventional or ultrasound hydrated grain showed no difference in pasting properties.

Keywords: High Power Ultrasound; Paddy Rice; Hydration

INTRODUCTION

High power ultrasound has affects mass transfer in a variety of systems, such as grain dehydration, osmotic dehydration and brining of meat and cheese[1]. Another area of food processing where ultrasounds effect on mass transfer may be of benefit is grain hydration. Hydration is an important process used to prepare grain for further processing (eg barley germination to make malt for beer; parboiling of rice). Hydration is a time consuming process, thus technologies to improve its efficiency could be of practical benefit.

The aim of this research was to investigate whether applying an ultrasound treatment during soaking of paddy rice at 50°C and 60°C alters the moisture content of the grain.

MATERIALS AND METHOD

Paddy rice from a short-grain variety (“Koshihikari”) was used in the experiments.

Conventional Hydration

Paddy rice (40 g) was weighed into a bag and pre-heated to test temperature in a waterbath. Water (100 ml) was placed in a beaker and adjusted to test temperature in a waterbath, then the rice was added to the water and the top of the beaker covered with aluminium foil to prevent evaporation. Thermocouples were placed in the sample, and the temperature logged. After hydration (20, 30, 60 or 120 minutes) the samples were drained on a sieve (aperture 500 µm) and the weight recorded. Moisture content was determined in triplicate by difference weighed after oven drying (130°C, 21 hours).

Ultrasound Hydration

A 24 kHz, 400 W ultrasound source was used in the trials (UP400S, Dr. Hielscher GmbH, Germany; fitted with a 22 mm diameter sonotrode). The ultrasound parameters were: 20% amplitude (20 µm) and 21% cycle pulse (ultrasound operated for 21% of each second). Paddy rice (40 g) was weighed into a bag and pre-heated to test temperature in a waterbath. Water (100 ml) was placed in a beaker and adjusted to the test temperature, then the rice was added to the water, and the ultrasound probe was immersed 10 mm below the surface of the water. At 60°C, temperature was maintained by heat produced by the ultrasound source. At 50°C, the beaker was placed in a waterbath to maintain the sample at 50°C. Two thermocouples were placed in the water and the temperature logged. After hydration
the samples were drained, weighed and the moisture content determined as described above.

RESULTS AND DISCUSSION

Ultrasound assisted hydration produced a greater increase in moisture content than conventional hydration at both 50°C and 60°C (Error! Reference source not found.). For example, at 50°C the water bath hydrated samples reached 29% moisture content after 120 minutes, while the ultrasound treated sample reached this moisture content in about 20 minutes. Since temperature was well controlled, it appears that non-thermal ultrasound effects are indeed accelerating the hydration of paddy rice.

While ultrasound increased moisture content at both temperatures, though the effect was greater at 50°C than 60°C. Based on the findings of previous studies, the more rapid hydration of rice grain at higher temperature is expected [2], which may also reduce the opportunity for ultrasound to enhance hydration. The lower effectiveness of ultrasound treatment at 60°C than 50°C may also be due to less efficient ultrasound cavitation, which is known to decrease with increasing temperature [1].

Figure 1. Moisture content of paddy rice hydrated at 50°C or 60°C in a waterbath, or with ultrasound assistance.

The rice grain surface is a barrier to water penetration into the inner parts of the grain. Microjets produced by ultrasound induced cavitation[1] could be increase hydration by damaging the grain surface layers. While no microscopic differences between ultrasound and conventional treatment were seen, the appearance of grains hydrated with the two processes is clearly different (Error! Reference source not found.). The surface of the ultrasound treated grain is smoother than the surface of the conventional treated grain. The difference in appearance may be partly due to hydration of the surface layers by ultrasound causing greater swelling and decreasing surface roughness. Perhaps more importantly, ultrasound treatment may abrade part of the grain surface layer by agitation or cavitation, effects that do not occur with conventional treatment. Ultrasound surface abrasion is supported by differences in the liquid recovered hydration. After conventional hydration the liquid is relatively clear, while after ultrasound hydration the liquid is darker with sediment present at the bottom of the beaker. This indicates that more solids from the grain have gone into the water during ultrasound treatment.
Since the volume of liquid remaining after conventional or ultrasound hydration differed (expected, since ultrasound treated grain had absorbed more water), a mass balance was performed to confirm the distribution of the dry matter Figure 3. It was shown that the liquid separated from the grain after ultrasound treatment represented a greater proportion of the total dry matter present than seen in liquid from conventional hydrated grain (2.0% and 0.5% respectively).

Microjets produced by ultrasound has also been shown to cleave polymer chains[3], thus ultrasound hydration may affect the ultimate functional properties of the rice starch. When RVA analysis was performed on starch extracted from rice grains after conventional or ultrasound treatment there was no significant different in their viscosity (ie peak, trough, final) during pasting.
CONCLUSION
Ultrasound treatment at 50°C and 60°C increases the moisture content of paddy rice compared to hydration in a waterbath. Ultrasound was more effective at 50°C than 60°C. Possible mechanisms for ultrasounds effect include: agitation of the sample, and abrading the surface to reduce the effectiveness of the surface layers as a barrier to water migration. Ultrasound treatment did not alter the pasting properties of starch extracted from ultrasound treated grains compared to conventionally treated grains.

REFERENCES


EMERGENT TECHNOLOGIES

Reynes Max

Dr Max Reynes have the following qualifications: Maîtrise de biochimie, TOULOUSE, food industries engineer from ENSAIA, Nancy and PHD from ENSAIA/INPL Nancy in food processing area (microwaves).

The presentation is above emergent technologies:

Good network relations between producers, industries and academics are necessary to determine how we can satisfy the needs of consumers. How then can research centres and networks provide solutions based on emergent technologies?

**Consumers and agro-industries worldwide** (Ref: Market study/2002/ France): 25% of consumers are looking for taste, $36 + 23 = 59\%$ are looking for health information (ESB, Pesticides, etc.), while 16% are concerned about a loss of quality from agro-industrial products.

**Consumers and food-industries** (Ref: Review/"60 millions of consumers" March 2004): consumers in the EU, in 2004, were asked the question “How well do we eat?” 70% answered less well than before! And to the question “What are the mean reasons why you say you eat less well than before?” the answer was because of agro-industry…

The new kinds of emergent technologies we have to take into account have to allow a preservation of healthy and organoleptic characteristics; limit browning reactions, and prevent neo-formed compounds. We have to adapt to the needs of the market (quality) and of consumers with often limited investment.

At Cirad we study and develop different innovative technologies in small scale units, such as frying processes coupled with osmotic dehydration; the flash expansion cooler for obtaining new fruit based products; membrane technology for juice stabilization & concentration without heating; (cold stabilization).
NEAR INFRARED EVALUATION OF TOTAL SOLUBLE SOLIDS AND DRY MATTER CONTENT IN DRAGON FRUIT (*Hylocereus undatus*)

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BASIC PRINCIPLES OF NEAR-INFRARED SPECTROSCOPY (NIRS)

Near-infrared spectroscopy has been used since the 1970s for the compositional analysis of low moisture food products. However, in the last ten years, the growing interest in near infrared (NIR) spectroscopy has arisen from improvements in instrumentation and data analysis. The NIR region of the electromagnetic spectrum range is of 780–2,526 nm. The most prominent absorption bands occurring in the NIR region are related to overtones and combinations of fundamental vibrations of C-H, O-H and N-H and S-H functional groups. This is fortunate for the analysis of food, since the major components water, lipid, carbohydrate and protein are well characterized by, –HOH, –CH2, –CHOH–, and –CONH— features respectively (Bokobza, 1998; Murray, 2004).

Among the advantages offered by NIR spectroscopy, the speed, the simplicity of sample preparation, multi-analysis and the non-destructive nature of the technique, its greatest disadvantage is the weak sensitive to minor constituents (less than 0.1%).

GENERAL USES OF NIRS IN FOOD INDUSTRY

In the baking industry NIR is used to analyze raw ingredients for protein content, moisture, particle size, ash, starch damage and color (flour color is directly related to the crumb color of a baked bread loaf). It is used to analyze process intermediates such as dough to determine a thorough mixing of ingredients of the presence/absence of an ingredient. NIR is also utilized with the final products, such as bread, pasta, biscuits and cereal, for fat, protein, moisture and egg content.

NIR has found its way into dairy industry as well. Used in the analysis of incoming milk in terms of fat, protein and lactose for payments to farmers and standardization of the milk in terms of fat, protein and solids. Spray-dried products are tested for fat, moisture and protein in order to get the proper composition mixture for special products and analysis of the raw materials for the production of cream, cheese, casein and whey. Again finish products are examined to control products in terms of legal regulations and customers’ of producers’ specifications.

The beverage industry has embraced NIRS applications for determining the constituents in alcoholic beverages such as beer, wine and distilled spirits, non-alcoholic beverages such as fruit juices, coffees, teas and soft drinks, and other products such as infant and adult nutritional formulas. It has also been used in determining the quality of the raw ingredients used to produce Chinese rice wine (Yu et al., 2006). NIRS can be applied to correlate the composition and sensory properties of tea (Yan, 2005), and even cheese product (Sinellie et al., 2005).

The application of NIRS in meat production also widely spread such as the predicting intramuscular fat content in pork and beef (Prevolnik et al., 2005) or the prediction the chemical composition of ground chicken carcasses and discriminate between carcasses from different genotypes (MsDevitt et al., 2005), or the detection of tenderness, post-rigor age and water status changes in sheep meat (McGlone et al.,, 2005).
USES OF NIRS FOR FRUITS AND VEGETABLE

The potential of near infrared spectroscopy (NIRS) to evaluate multiple attributes of fruits has been demonstrated repeatedly in the literature (Zude, 2003; Slaughter et al. 1996; Saranwong et al. 2004; Walsh et al., 2004). The most often quality attributes have being determined are total soluble solids (TSS), dry matter content (DMC), starch, and total titratable acidity in a wide range of fruits including pineapple (Shiina et al. 1991; Guthrie et al., 1998); kiwi (McGlone et al., 1998); apple, banana, peach (Walsh et al., 2004; Kawano et al., 1995; McGlone et al., 2003); mango (Saranwong et al., 2003); avocado (Clark et al., 2003); nectarine, plum (Golic et al., 2006); apricot (Manley et al., 2007); and cucumber (Kavdir et al., 2007).

Beside of those attributes, the detection of fruit disorder are also emphasized as and brown hear in pears (Zerbini et al., 2002); chilling injury and rots in kiwi (Clark et al. 2004); and the translucent flesh disorder in intact mangosteen (Teerachaichayut et al., 2007). Pectin constituents in Japanese pear are other contributes that evaluated (Sirisomboon et al., 2007).

Some attempt has been made to construct the harvesting index for Vietnamese dragon fruit (Hylocereus undatus) based on days after flowering (Hoa, et al, 2000) in which the difference between maturity stages were indicated by skin colour changes. Nevertheless, during 5 days (from 25 days to 30 days after flowering) the skin colour changed rapidly (Hoa et al., 2002) which is obviously intricate to make a difference between fruit maturity stages. In addition there is no available study on dragon fruit in regarding to the application of near infrared technology to non-destructively evaluate the fruit’s internal quality.

SIGNIFICANT AND OBJECTIVES OF THE STUDY

The success of model building depends strongly on the kind of fruit and its geometrical, physical and chemical characters. Models for the determination of totalsoluble solids and dry matter content of fruits with a thin skin are often good with high $R^2$ varying between 0.85 - 0.96, low RMSEP varying between 0.42 - 1.11% and SDR greater than 2. Such fruits include tomato (Slaughter et al., 1996), peach (Kawano et al., 1995), apricot (Paolo et al., 2000; Manley et al., 2007), mango (Saranwong, 2003), avocado (Clark et al., 2003) and grape (Arana, 2005). In contrast, fruits with a thick skin are more challenging and models are generally poorer. Examples include durian (Yantarasri et al., 2000) ($R^2 = 0.74$ for sugar analysis, no report of other performance parameters), mangosteen (Teerachaichayut et al., 2007) and water melon (Tian-Hai, 2006). However, there are other fruits with thick skin for which good models have been produced, for example, TSS of banana ($R^2 = 0.98$, SEP = 0.8) (Tarkosova et al., 2000), TSS of melon ($R^2 = 0.87$, SEC=1.6) (Long et al., 2002) and Satsuma mandarin ($R^2 = 0.989$, SEC=0.28) (Kawano et al., 1993), and conversely thin skinned fruit such as pickling cucumbers for which the dry matter prediction model was poor ($R^2 = 0.39$, SEP = 0.33) (Kavdir, 2007). Apart from fruit geometrical, physical and chemical characters, the wavelength region selected, which can be associated with specific fruit constituents, can influence prediction model performance (Paolo, 2000).

Non-related spectral variation that derives from the fruit geometrical, physical and chemical characters can be reduced by pre-treating the spectral data prior to model building. The selection of informative wavelengths is also considered to be a crucial step prior to the construction of a quantitative calibration model (Skibsted et al., 2004). Nevertheless, there is no widely accepted way of selecting which pre-treatment method or wavelengths to use, or of how to combine methods. Thus the objectives of this study were (i) to develop a procedure to optimize the use of spectral data pre-treatments, and then (ii)
to demonstrate the feasibility of building NIRS prediction models for the determination of total soluble solids (TSS) and the dry matter content (DMC) of dragon fruit.

RESULTS
The use of derivatives of absorbance data which are typically used to avoid influences from changes in the baseline of spectral data, generally greatly improved model performance compared with raw absorbance data. It was efficient for spectra data pretreatment in developing TSS prediction models in the wavelength range 750-1,080 nm. Meanwhile MSC and SNV, which both minimize additive and multiplicative effects of the fruit samples, were useful in developing DMC prediction models in the wavelength range 800-1,000 nm.

REFERENCES


KINETIC MODELING OF 5-METHYL AND 5-FORMYLTETRAHYDROFOLIC ACID DEGRADATION DURING TEMPERATURE AND HIGH PRESSURE TREATMENTS

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INTRODUCTION

The use of high pressure in industrial applications requires the identification of optimal pressure/temperature/time combinations resulting in a limited quality degradation within the constraints set by the target microbial/spore inactivation. The aim of this investigation is to systematically study the stability of 5-methyltetrahydrofolic acid (5-CH₃H₄PteGlu) and 5-formyltetrahydrofolic acid (5-CHOH₄PteGlu) in model systems on a kinetic basis during high pressure thermal (HP/T) processing in comparison with conventional thermal (T) treatments.

MATERIALS AND METHODS

Sample preparation

The stock solutions of 5-CH₃H₄PteGlu and 5-CHOH₄PteGlu (>95% purity, Switzerland) were prepared and stored at -80°C. Working solutions (5-CH₃H₄PteGlu and 5-CHOH₄PteGlu) of 10µg/mL were daily prepared respectively in sodium phosphate buffer (pH 7) and in sodium acetate buffer (pH 5) for T or HP-T treatments.

Thermal and high pressure degradation kinetics

The kinetic experiments were performed in the HP/T range from 100-800 MPa/20-70°C. 5-CH₃H₄PteGlu and 5-CHOH₄PteGlu samples were enclosed in 0.3 mL flexible microtubes. After reaching the desired pressure, the individual vessels were isolated and the pressure was maintained in the vessels until the valves were opened. The time course of the experiment (time=0) was started after reaching the equilibration period (2 min). At this moment, the 1st pressure vessel was decompressed and the residual concentration of the sample in the corresponding vessel was considered as blank. The other vessels were then decompressed as a function of time. After withdrawal, the samples were stored in an ice bath and subsequently the residual vitamin concentration was measured.

Data analysis

Kinetics of 5-CH₃H₄PteGlu and 5-CHOH₄PteGlu degradation were analyzed using a first order reaction kinetic model. The temperature and pressure dependence of the $k$-values were determined respectively using Arrhenius (Eq. 1) and Eyring (Eq. 2) relationships.

\[
k = k_{ref} \exp \left[ \frac{E_a}{R} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right) \right]
\]

\[
\ln(k) = \ln(k_{ref}) - \frac{V_a}{R T_{ref}} (P - P_{ref})
\]

The kinetic model (Eq. 3) derived from the thermodynamic model of Hawley (1971). This equation was used as a starting point to describe the experimentally obtained 5-CH₃H₄PteGlu and 5-CHOH₄PteGlu degradation data.

\[
\ln(k) = \frac{A}{T} (P - P_{ref})^2 - \frac{B}{T} (P - P_{ref}) + \frac{C}{T} (T - T_{ref}) + \frac{D}{T} \left[ \ln \left( \frac{T}{T_{ref}} \right) - 1 \right] + T_{ref} \left[ \ln \left( \frac{T}{T_{ref}} \right) - 1 \right] + \ln(k_{ref})
\]
Where $P$ is the pressure (MPa), $T$ is the absolute temperature (K), $P_{\text{ref}}$ and $T_{\text{ref}}$ are the reference pressure (MPa) and temperature (K), respectively, $k$ is the inactivation rate constant (min$^{-1}$). A, B, C, D and E are physical parameters.

RESULTS AND DISCUSSIONS

**Pressure degradation kinetics of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu**

In the pressure temperature area studied, it was observed that the degradation of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu due to combined HP/T conditions follows first order kinetics. The iso-degradation rate contour diagrams of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu as function of HP-T are depicted in Fig. 1. These results show an effect of HP/T on 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu degradation at all HP/T combinations tested and the same degradation rate constant can be achieved by selecting different HP/T combinations.

(a) The inner and outer lines represent HP/T combinations for $k$-value equal to 0.0075 and 0.0115 min$^{-1}$, respectively

(b) The inner and outer lines represent HP/T combinations for $k$-values equal to 0.003 and 0.005 min$^{-1}$, respectively

**Figure 1.** HP/T kinetic diagram for the pressure temperature degradation of (a) 5-CH$_3$H$_4$PteGlu in phosphate buffer and (b) 5-CHOH$_4$PteGlu in acetate buffer.

**Table 1.** $E_a$ values (kJ/mol) for thermal degradation of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu (10µg/mL) at atmospheric and elevated pressures ("SE of regression, nd: not determined"

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>5-CH$_3$H$_4$PteGlu (pH 7)</th>
<th>5-CHOH$_4$PteGlu (pH 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>79.98± 4.88$^a$ ($r^2$ = 0.99)</td>
<td>62.81± 3.56$^a$ ($r^2$ = 0.99)</td>
</tr>
<tr>
<td>100</td>
<td>78.97± 24.16 ($r^2$ = 0.91)</td>
<td>nd</td>
</tr>
<tr>
<td>200</td>
<td>71.88± 7.95 ($r^2$ = 0.98)</td>
<td>58.75± 5.88 ($r^2$ = 0.98)</td>
</tr>
<tr>
<td>300</td>
<td>nd</td>
<td>77.15± 13.62 ($r^2$ = 0.94)</td>
</tr>
<tr>
<td>400</td>
<td>81.09± 5.39 ($r^2$ = 0.99)</td>
<td>52.18± 2.49 ($r^2$ = 0.99)</td>
</tr>
<tr>
<td>500</td>
<td>nd</td>
<td>51.59± 5.03 ($r^2$ = 0.97)</td>
</tr>
<tr>
<td>600</td>
<td>100.18± 11.89 ($r^2$ = 0.96)</td>
<td>53.39± 3.60 ($r^2$ = 0.99)</td>
</tr>
<tr>
<td>800</td>
<td>90.10± 3.30 ($r^2$ = 0.99)</td>
<td>60.55± 5.06 ($r^2$ = 0.98)</td>
</tr>
</tbody>
</table>

The $E_a$ values of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu degradation at different pressure levels were estimated by a linear regression analysis (Table 1) and the linearized Arrhenius model (Eq. 1) was valid over the entire HP-T domain studied. The pressure dependence of the degradation rate constants at constant T is estimated using the Eyring model (Eq. 2) and the estimated values of $V_a$ at constant T are presented in Table 2. At all T levels tested, the estimated activation volumes showed a negative sign, indicating an acceleration of the 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu degradation by increasing P.
Mathematical model to describe the combined pressure and temperature dependence of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu degradation rate constants:

On the basis of the estimated kinetic data, Eq. 3 was used as a starting point to describe the combined HP-T dependence of experimentally obtained 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu high pressure-temperature degradation rate constants. When the whole data set of folate degradation was analysed using Eq. 3, $V_a$ is independent of T and HP [E=0 when $V_a$\(\neq f(T)\) and $V_a$\(\neq f(P)\)]. The results shown in Table 2 illustrate only small changes of $V_a$ with T. Moreover, $E_a$ and $V_a$ are independent, respectively on T [D=0 when $E_a$\(\neq f(T)\) and on P [A=0 when $V_a$\(\neq f(P)\)]. Assuming that E=0, A=0 and D=0, Eq. 3 reduces to Eq. 4. In this study, Eq. 4 was applied to describe the combined HP/T dependency of the 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu degradation rate.

$$
\ln(k) = -\frac{B}{T}(P-P_{ref}) + \frac{C}{T}(T-T_{ref}) + \ln(k_{ref}) \tag{4}
$$

Table 2. Estimated activation volumes $V_a$ (cm$^3$.mol$^{-1}$) for isobaric-isothermal degradation of 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu (\textit{SE of regression; nd: not determined})

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>5-CH$_3$H$_4$PteGlu (pH 7)</th>
<th>5-CHOH$_4$PteGlu (pH 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>-5.79 ± 0.19$^a$ (r$^2$ = 0.99)</td>
<td>Nd</td>
</tr>
<tr>
<td>40</td>
<td>-5.24 ± 1.39 (r$^2$ = 0.90)</td>
<td>-3.37 ± 1.05$^a$ (r$^2$ = 0.91)</td>
</tr>
<tr>
<td>50</td>
<td>-7.05 ± 0.88 (r$^2$ = 0.96)</td>
<td>-8.29 ± 1.23 (r$^2$ = 0.92)</td>
</tr>
<tr>
<td>60</td>
<td>-7.23 ± 0.67 (r$^2$ = 0.94)</td>
<td>-10.50 ± 1.24 (r$^2$ = 0.95)</td>
</tr>
<tr>
<td>65</td>
<td>-10.67 ± 0.38 (r$^2$ = 0.99)</td>
<td>-9.14 ± 0.61 (r$^2$ = 0.98)</td>
</tr>
<tr>
<td>70</td>
<td>Nd</td>
<td>-12.42 ± 0.65 (r$^2$ = 0.99)</td>
</tr>
</tbody>
</table>

In the HP/T combinations studied, a satisfactory correlation between the predicted ln($k$) and the experimentally determined ln($k$) was found (Fig. 5). By inserting the estimated model parameter values into Eq. 4, the simulated HP/T combinations resulting in the same degradation rate constants for 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu were depicted in Fig. 6.

Figure 5 Correlation between the experimentally determined ln($k$)-values and the ln($k$)-values estimated for (a) 5-CH$_3$H$_4$PteGlu & (b) 5-CHOH$_4$PteGlu using the model described in Eq. 4
The inner and outer lines represent $k$ values equal to 0.0075 and 0.0115 min$^{-1}$, respectively.

The inner and outer lines represent $k$ values equal to 0.003 and 0.005 min$^{-1}$, respectively.

**Figure 6.** Predicted HP/T kinetic diagram for HP/T degradation of (a) 5-CH$_3$H$_4$PteGlu and (b) 5-CHOH$_4$PteGlu by inserting the estimated model parameters into Eq. 4

**CONCLUSIONS**

On the basis of the estimated $k$-values obtained in this study, it is obvious that 5-CHOH$_4$PteGlu (pH 5) is more HP/T stable than 5-CH$_3$H$_4$PteGlu (pH 7). Regarding to the use of HP technology in commercial pasteurization, pressurization (<400 MPa) at moderate T (<40°C) for a short period (<30 min) can maintain the 5-CH$_3$H$_4$PteGlu and 5-CHOH$_4$PteGlu concentration. The formulated mathematical models, expressing the $k$-values as a function of HP/T can provide a tool to design, assess and optimize combined HP/T processes.
FUNCTIONAL PROPERTIES OF EDIBLE BILAYER FILMS COMPOSED OF POLYSACHARIDES AND SHELLAC FOR FOOD QUALITY PRESERVATION

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ABSTRACT

Edible bilayer films composed of agar (AG) or cassava starch (CAS) as a cohesive structural layer and shellac as a moisture barrier are investigated for their potential use in food preservation. Bilayer films with unplasticized shellac exhibit low water vapor permeability (WVP) \((0.89 – 1.03 \times 10^{-11} \text{g} \times \text{m}^{-1} \times \text{s}^{-1} \times \text{Pa}^{-1})\) and a quite hydrophobic surface. However, the shellac layer tends to be cracked and scaled off. The incorporation of PEG 200 as plasticizer into shellac could improve the flexibility that prevents the rigidity and brittleness of shellac and therefore, strengthen the adhesion between shellac and the polysaccharide layer. However, the use of plasticizer in shellac could affect slightly the WVP of the films. Either being plasticized or not, shellac layer could improve significantly the functional properties of edible films and give a promising use for food preservation.

Keywords: edible bilayer film; agar, cassava starch, shellac, film microstructure, water vapor permeability, surface hydrophobicity

INTRODUCTION

The loss of product shelf life is generally determined by quality and safety factors such as microbial stability, chemical changes, physical and sensorial properties. These factors all depend on moisture content and water activity (aw) of food product (1). According to Kester & Fennema (1986) (2), there are the critical values of aw for microbial proliferation, changes in food texture, chemical deterioration and enzymatic reactions. Therefore, one of most important functional properties of edible films or coatings is the resistance to moisture migration between the surrounding medium and food products.

Many researches on functional properties of edible films showed that proteins and polysaccharides based films have quite high water vapor permeability (WVP) compared to edible waxes or resins based films (2, 3, 4, 5). However, most of hydrophobic compounds can not form sufficiently cohesive films. Therefore, edible bilayer films composed of proteins or polysaccharides and lipids have been developed. In the majority of studied models, the hydrophobic compound is usually fatty acids, hydrogenated vegetable oils or natural waxes (6, 7, 8, 9, 10, 11, 12). However, the "poor" sensory characteristics such as greasy surface, waxy taste and rancidity make their use in food coating to be limited. Moreover, another way to improve moisture barrier efficiency of edible films and coatings is the application of edible lacs and varnishes. Currently, in food and pharmaceutical industries, shellac is known as enteric material that provides a protective layer for active substances as well as a good moisture barrier and desirable gloss. Shellac was usually used alone in hard confectionery coating or in mixture with lipid compounds in fruit coatings (13, 14). Despite the wide use of shellac as coating, there is very few information relative to edible bilayer shellac-hydrocolloid films.

The efficiency against moisture transfer of shellac and the potential use as edible packaging of CAS and AG deal with the investigation on new applications of these
The aim of this work is to improve the moisture barrier properties and surface hydrophobicity of edible films made from CAS and AG spreading with shellac. At the same time, the influence of polyethylene glycol (PEG) on functional properties of shellac layer will be studied.

MATERIALS AND METHODS

Materials
Agar (AG) (food quality, Ha Long Co, Vietnam), cassava starch (CAS) (food grade, Tai Ky food-enterprise, Vietnam), were used as the film-forming component to provide a continuous matrix of edible film. Anhydrous glycerol (98% purity, Fluka Chemical, Germany) was added to improve their mechanical properties. Shellac (C.E. Roeper GmbH, Germany) was used as hydrophobic component providing water barrier layer. Polyethylene glycol 200 (M = 190-210g.mol\(^{-1}\), Merck, Germany) was used to improve flexibility of shellac. Two synthetic films were also used as standards: a cellophane film (300P, Courtauld’s, U.K.), hydrophilic but not soluble in water, and a low density polyethylene film which considered as hydrophobic film (LDPE, Riblène FF30, EniChem, France). All the test films are considered as dense and then non porous.

Preparation of edible films

Homogeneous edible films: Agar films were obtained after solubilization of 3g of AG in 100ml of osmosed water at 95°C for 30min under a 600-rpm magnetic stirring. Then, glycerol was added at a concentration of 15% of the total dry basis. The film-forming solution was kept for 10min under the same conditions of temperature and stirring prior to be spread onto a glass plate that had been previously covered with polyvinyl-chloride adhesive sheet to prevent the sticking of dried films. A continuous thickness of 1000µm was applied for the cast solution using a thin-layer chromatography spreader. All the films were dried for 5h in a ventilated cupboard (KBF 240 Binder, ODIL, France) with temperature and relative humidity fixed at 40°C and 30%RH, respectively. The CAS films were prepared with the same procedure except that the film-forming solution was composed of 5g in 100mL of water.

Bilayer edible films: The solution of shellac (SH) was prepared by dissolving 30g shellac flakes in 100mL of ethanol 98% under magnetic stirring. In the case of plasticized shellac (SHP), 4.5g of PEG 200 were added after the shellac flakes were completely dissolved. The bilayer films were obtained by depositing a thin layer of shellac solution onto the surfaces of dried preformed CAS or AG based films. The composite films were then placed in a ventilated oven at 30°C to evaporate the ethanol. To study the effect of shellac layer thickness on the WVP of bilayer films, 50µm and 30µm thickness of shellac solution were applied onto AG based films.

Characterization of the films

Film thickness was determined by using an electronic gauge (Multicheck FE, SODEXIM, France) with precision ranges between 0.1 and 1% as a function of thickness value (0-100µm or 0-1000µm). Ten replicates were done on each film-making.

Film microstructure was observed by Environmental Scanning Electron Microscopy (ESEM, Phillips XL 30 ESEM, Japan). A 5x10 mm\(^2\) film was fixed on the support using double side adhesive tape, with an angle of 90° to the surface, which allowed observing the film cross section. Any particular film preparation was necessary.

Water vapor permeability (WVP) at a relative humidity differential of 22-57%; 22-84% and 22-99% was measured using a modified French standard method (15), homologous to the ASTM E96-80 (1980) (16) method and adapted to edible materials by Debeaufort et
al., (1993) (17) and McHugh et al., (1993) (18). Prior measuring WVP, all film samples were equilibrated at 25°C for 48h in desiccators over potassium acetate saturated solution (CH₃COOH, Merck, Germany) which fixed RH at 22%. Films samples were then fixed between two Teflon rings on the top of the glass cell containing a saturated salt solution of sodium bromide; potassium chloride (NaBr; KCl, Merk, Germany) or distilled water of which the water activity is 0.57; 0.84 and 0.99 respectively at 25°C. Test cell was introduced into a climate-controlled chamber (KBF 240 Binder, ODIL, France) regulated at 22%RH and 25°C. Test cell was periodically weighted up to a constant weight variation rate. WVP (g×m⁻¹×s⁻¹×Pa⁻¹) was calculated using the following equation:

\[
WVP = \frac{\Delta m \times x}{A \times \Delta t \times \Delta p} = \frac{WVTR \times x}{\Delta p}
\]

Where \(\Delta m\) is the weight loss (g) of the test cell, \(x\) is the film thickness (m) with \(A\) is exposed area (8.11×10⁻⁴ m²) during \(\Delta t\) duration (s) under \(\Delta p\) partial water vapor pressure (Pa), and \(WVTR\) is the water vapor transfer rate (g×m⁻²×s⁻¹).

**Surface hydrophobicity and wet ability** of the film were evaluated from the contact angle measurement of water droplet deposited on the film surface using a G1 Krüss goniometer (KRÜSS GmbH, Germany) equipped with a CCD camera and an image analysis software (Drop Shape Analysis DSA5.0, KRÜSS, Germany). Kinetics of contact angle and droplet volume was determined. Prior measuring, all films samples were conditioned at 57% RH and 25°C.

**Mechanical properties** were evaluated by tensile strength at breaking (TS) and percentage of elongation (%E) was measured using a Universal Testing Instrument (Instron UTII 1122, Instron Ltd.) with a 5kN load cell. Samples were stored at 57%RH over the sodium bromide saturated solution for 10 days at 25°C prior measurement. A total of 20 samples for each type of films were stretched at a constant rate of 100mm×min⁻¹. The effective dimension of the film before the stretching was 20×60mm².

**Significance.** Each film attribute was measured at least in triplicate, and the differences between means were tested at p<0.05 level using Statgraph software.

**RESULTS AND DISCUSSION**

On macroscopic observations, both agar-shellac (AG-SH) and cassava-shellac (CAS-SH) bilayer films have the yellow color originating from shellac. Even if they have been deposited onto a structural support, un-plasticized shellac layers of composite films are rigid, tend to be fissured and scaled off. These disadvantageous characteristics of shellac are visibly improved when PEG 200 was added, the films become more flexible and easy to handle.

**Film microstructure**

The figure 1 displays the environmental scanning electron microscopy (ESEM) observations of the cross section of the films. The two parts of bilayer films can be distinctly observed, the upper part displays the structure of shellac layer and the lower exhibits the base support, which is cassava starch (CAS) or agar (AG) based film. For un-plasticized shellac, the cross sections with some small cracks and pores (figures 1b and 1e) were observed which permit to assume a heterogeneous and irregular structure. Furthermore, a disjunction is remarkably visible between shellac layers and the base films. Inversely, as showed in figures 1c and 1f, addition of PEG 200 into shellac leads to the more homogeneous structure. Moreover, the adhesion between the two layers is perceptibly improved. According to Limmatvapirat et al. (2004) (19), the major part of shellac is composed of hard resin corresponding to crystalline region. Such structure leads
to rigid and brittle characteristics of shellac layer. As a small compound, PEG 200 can penetrate in shellac matrix and thus increasing film flexibility. Moreover, PEG is able to attract water that can amplify the plasticizing effect.

Figure 1: ESEM cross section micrographs of the films: (a) CAS monolayer, (b) CAS-shellac bilayer, (c) CAS-plasticized shellac bilayer, (d) AG monolayer, (e) AG-shellac bilayer, (f) AG-plasticized shellac bilayer (double arrows indicate film thickness; 1: shellac layer; 2: edible base film layer)

The mechanisms of interfacial adhesion in multilayer system are numerous and depend on polymer nature. Theoretically, PEG is neither copolymer nor amphiphilic molecules that play a surface-active role. The differences in nature and the lack of a copolymer at the interface explain the disjunction between unplasticized shellac layer and the base films. Moreover, Felton et al. (1997) (20) reported that adhesion properties of polymeric films depend directly on their elasticity. Therefore, the adhesion between shellac and base layer could be the result of the plasticizing effect of PEG 200 on the shellac elasticity.
The figure 1 shows also that after coating with shellac, the structure of CAS layer is distinctly changed. The reason could be the effect of ethanol on the starch. During the lamination of shellac solution, ethanol apparently penetrated into the polymeric matrix of CAS layer, inducing thus an increase of crystalline degree of amylose component in the film and then modifies strongly CAS film structure.

**Water vapor permeability of films**

The moisture barrier efficiency of edible films is usually evaluated from the measurement of water vapor permeability (WVP). The RH gradients chosen in present work are 22-57%; 22-84% and 22-99%, they represent the quite severe RH conditions between product and external environment or between different compartments in a composite food. The WVP of monolayer films and bilayer films are reported in table 1.

Although the moisture barrier efficiency of bilayer films studied is still lower than that of LDPE film, the WVP of AG and CAS films are remarkably reduced when shellac layer is applied.

The plasticizer using to improve the flexibility of shellac layer has an effect on the moisture barrier properties of bilayer films. Generally, WVP of bilayer films composed with plasticized shellac layer are more sensible to environmental RH gradients than those without plasticizer. It is well know that incorporation of plasticizer into a polymer matrix decreases the interactions between molecular chains, increases also the free volume, hence water molecules are diffused more easily that conducing to a higher WVP (21). However, in the case of bilayer films covered with a thin layer of shellac (–SH30 and –SHP30), whether the films contained plasticizer or not, no differences significant in WVP are observed. The moisture barrier properties of edible bilayer films in this case depend on the film structural integrity rather than on the plasticizer used for shellac. As showed in figure 1, unplasticized shellac structure displays some defects (small cracks and pores), which could affect the WVP of the films if the thickness is not sufficient.

**Surface hydrophobicity and wetability**

Contact angle of a water droplet deposited onto the films were used to estimate the surface hydrophobicity and wet ability of edible films and coatings. The experimental values are displayed in table 1, in which initial contact angle was obtained immediately after the deposition of water droplet (at t = 0.5 sec) and adsorption rate was calculated from droplet volume kinetic. For all bilayer films, initial contact angle values vary from 76 to 93°; although they are still significantly lower than that of LDPE film (≈ 105°), these values indicate the quite hydrophobic surface of shellac layers. Several studies have been pointed out the surface hydrophobicity of enteric coatings for pharmaceutical tablets by using food-grade lacs or resins. They have been showed that plasticized shellac used for cetamol pellet coating exhibited a contact angle value around 65-70° (45), and that of composite acrylic resin-wax was 80-85° (20). It could be assumed that the surface hydrophobicity of bilayer films in this work is considerably improved by the shellac layer. This is confirmed by the significant increase in contact angle of CAS-SH bilayer film compared to monolayer CAS based films.
Table 1: Water vapor permeability ($10^{-11}$g·m$^{-1}$·s$^{-1}$·Pa$^{-1}$) at 25°C, contact angle (°) and water adsorption rate ($\times 1000\mu$L·s$^{-1}$) of edible films based on agar (AG); cassava starch (CAS) and their bilayer films coated with shellac (SH) compared to those of cellophane and low density polyethylene (LDPE) films.

<table>
<thead>
<tr>
<th>Composition of film</th>
<th>Thickness (µm)</th>
<th>Water vapor permeability ($10^{-11}$g·m$^{-1}$·s$^{-1}$·Pa$^{-1}$)</th>
<th>Contact angle (°)</th>
<th>Water adsorption rate ($10^{-3}$µL·s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\Delta$RH: 22-57%</td>
<td>$\Delta$RH: 22-84%</td>
<td>$\Delta$RH: 22-99%</td>
</tr>
<tr>
<td>[AG + 15% glycerin]</td>
<td>37.38 ± 1.17</td>
<td>7.21 ± 0.32 c</td>
<td>12.52 ± 0.30 e</td>
<td>13.70 ± 0.94 d</td>
</tr>
<tr>
<td>[CAS + 15% glycerin]</td>
<td>47.77 ± 5.81</td>
<td>5.58 ± 0.24 d</td>
<td>11.19 ± 1.00 d</td>
<td>11.22 ± 0.70 c</td>
</tr>
<tr>
<td>[AG + 15% glycerin]+[SH]$_{30}$</td>
<td>[26.29+37.38]±3.99</td>
<td>0.38 ± 0.08 ab</td>
<td>4.83 ± 0.74 c</td>
<td>3.05 ± 0.11 b</td>
</tr>
<tr>
<td>[AG + 15% glycerin]+[SH]$_{50}$</td>
<td>[41.73+37.38]±3.58</td>
<td>0.35 ± 0.01 a</td>
<td>0.89 ± 0.15 a</td>
<td>1.03 ± 0.14 a</td>
</tr>
<tr>
<td>[AG + 15% glycerin]+[SH+PEG]$_{30}$</td>
<td>[14.00+37.38]±4.62</td>
<td>1.14 ± 0.11 c</td>
<td>2.89 ± 0.96 b</td>
<td>3.18 ± 0.82 b</td>
</tr>
<tr>
<td>[AG + 15% glycerin]+[SH+PEG]$_{50}$</td>
<td>[27.67+37.38]±3.95</td>
<td>0.68 ± 0.01 bc</td>
<td>2.49 ± 0.08 b</td>
<td>2.12 ± 0.17 ab</td>
</tr>
<tr>
<td>[CAS + 15% glycerin]+[SH]$_{50}$</td>
<td>[36.79+47.77]±4.68</td>
<td>1.13 ± 0.64 c</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>[CAS + 15% glycerin]+[SH+PEG]$_{50}$</td>
<td>[21.59+47.77]±2.91</td>
<td>0.90 ± 0.42 bc</td>
<td>2.32 ± 0.08 b</td>
<td>2.91 ± 0.40 b</td>
</tr>
<tr>
<td>Cellophane</td>
<td>22.00 ± 0.41</td>
<td>4.54 ± 0.13</td>
<td>7.94 ± 0.25</td>
<td>8.37 ± 0.07</td>
</tr>
<tr>
<td>LDPE</td>
<td>30.00</td>
<td>n.d.</td>
<td>0.19 ± 0.01</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Values in the same column followed by the same letter are not significantly different at p<0.05 level; n.d.: not determined.
Mechanical properties
Tensile strength (TS) and percentage of elongation (%E) at break of stretched samples were determined to estimate the effect of shellac layer on the mechanical properties of AG and CAS based films. The TS and %E are displayed in figure 2. Compared to the TS of LDPE film, which varies from 9 to 17MPa (5), the TS of bilayer films based on CAS and shellac are in the same order, those of AG-SH are significantly higher. The results show also that application of shellac coating tends to reduce significantly the TS of AG and CAS based films. However, in these cases, the results do not allow displaying the effects of shellac layers on mechanical property changes of bilayer films. Indeed, the TS values should be decreased with increasing of film thickness if the tensile force obtained remains constant. Consequently, the TS of bilayer films in this work should be decreased due to the increase of film thickness (AG or CAS layer + shellac layer) while tensile force of base film layer remain unchanged. During the stretching test, unplasticized shellac layers were always broken very early prior the total breaks of bilayer films. The figure 2 shows also that %E of bilayer films is modified by the coated with shellac layer. Coating with unplasticized shellac, the %E of both AG and CAS based films are slightly decreased. The reason could be the influence of rigid and brittle characteristics of shellac on the stretchability of bilayer films.

Figure 2: Tensile strength (TS) and percentage of elongation (%E) for monolayer AG, CAS based films and their bilayer films coated with shellac (values followed by the same letter are not significantly different at p<0.05 level).

CONCLUSIONS
The application of shellac onto AG or CAS based films could enhance their moisture barrier efficiency but the adhesion between these two layers is poor. Addition of PEG 200 as plasticizer could improve significantly the flexibility of shellac which prevents the defects in their structure, and the disjunction between shellac layer and base film layer. The efficiency against moisture transfer of AG and CAS based films are considerably improved with the shellac layers. In the same manner, shellac coating improves significantly the resistance to liquid water transfer. The surfaces of shellac layers are considered as hydrophobic with quite high values of contact angle. The tensile strength of bilayer films seems to be affected by the application of shellac layer. However, this influence is due mainly to the increase in films thickness, whereas the tensile force remains constant. The incorporation of PEG 200 into
shellac layer allow increasing the stretch ability of bilayer films compared to monolayer AG and CAS based films.

The enhancement in moisture and liquid water barrier efficiency, the reduction in moisture sorption as well as the ability to maintain mechanical properties of hydrocolloid-shellac bilayer films give a promising utility for food packaging or coating. It should be noticed that organic solvents used to dissolve shellac has proved disadvantage for some hydrocolloid base layers such as starch, the substitution of ethanol by another solvent or shellac dispersion in aqueous solutions would an interesting investigation for edible films and coatings.

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mechanical properties, water resistance and aging of thermo-moulded films made from
A laboratory scale microwave vacuum evaporator (MVE) was designed and developed. The performance was evaluated by determining the effects of microwave power outputs at 575, 428 and 321 watts and vacuum degrees at -80, -70 and -50 kPa on syrup concentration. The results showed that microwave power outputs were highly significant in exponential form with the concentration and slightly significant with vacuum degree. Microwave vacuum and rotary vacuum evaporator (RVE) were compared in term of concentrating rates and color quality parameters of pineapple juice. Results revealed that the rate of concentration for pineapple juice by MVE was 2.5 times faster than RVE. In term of color quality parameters of pineapple juice, results revealed that color change by MVE was 1.7% while color change by RVE was 8.55%.

INTRODUCTION
The concentration of fruit juices using multi-stage vacuum evaporation provides a reduction of transport, packaging and storage costs. In addition, the concentrates are more stable, presenting a higher resistance to microbial activity than the original juice in similar conditions and also enabling the compensation of changes in quality, quantity and price of the fruits during harvests. For concentration process, there is a significant loss of aroma compounds in the first minutes of operation leading to an irreversible alteration of the aroma profile of the juice (Petrotos and Lazarides, 2001). Additionally, there is a color change and a reduction of the nutritional value due to the high temperatures of operation (Alves and Coelho, 2006).

Many efforts have been devoted to develop improved methods such as freeze concentration, sublimation concentration and membranes (ultra filtration and reverse osmosis) for concentrated juice processing. One of the solutions to this problem is the use of alternative processes that avoid high temperatures of operation by using microwave vacuum, which, suite the requirement for optimal concentration process including: rapid rate of heat transfer, low temperature operation through application of a vacuum, efficient vapor liquid separation and efficient energy use and recovery (Helaman, 1997). Many research on the quality of dried banana slices, cranberries, oregano and potato cubes under microwave vacuum were studied by Schubert and Drouzas (1996), Yongsawatdigul and Gunasekaran (1996b), Yousif et al (2000) and Bondaruk et al. (2007), was examined. The results showed the better retention of key constituents and sensory properties than its air-dried counterpart. Yongsawatdigul and Gunasekaran (1996a) also remarked that the microwave-vacuum drying technique has been successfully applied to numerous food materials. However, though there have been many studies using Microwave vacuum in drying process, the study of microwave vacuum in evaporation process is limited. Therefore, this study was undertaken to investigate the effect of power levels and vacuum operating pressure degree on evaporation rate and compare the color loss of concentrate pineapple juice (microwave vacuum evaporator (MVE) and rotary vacuum evaporator (RVE) processes were also compared).
Design and development laboratory scale of MVE

The microwave vacuum evaporator was used in the experiment is shown in Fig 1. This microwave vacuum system consisted of a 750 kW power generator and a multimode stainless steel microwave cavity (55 cm x 30 cm x 35 cm) Model IV-5 (Turbolar, Industrial Microwave Generator). The power unit transformed a 220 V power supply to the 1000 V required by the magnetron. Stability and control of the power output was maintained by the feedback of a 4–20 mA, control signal from the magnetron anode current. Power meters and other control instrumentation monitored microwave power input to and reflected power from the cavity. Uniformity of the microwaves in experiment the cavity was enhanced by adjustable stirrer (0 to 200 rpm). The capabilities of the vacuum pump was -50 kPa to -1000 kPa and vacuum pressure was adjustable by vacuum regulator (SMC model T203-1-20G, Japan) which had high stable condition.

Modified rotary vacuum Model EL 131 and the vacuum pump Model B-169 (Buechi, Switzerland) were combined with microwave oven. The vacuum system was controlled and adjusted by vacuum regulator, Model T 203-1-20G (SMC, Japan). For the control system, PLC model vision 120 (Unitronics, USA) was used as a microwave power input controller and it also used to record data. The unit was connected to the computer over a RS-232 interface and then analyzed the data by visililogic program version 4.60.

![Fig 1. Schematic of the microwave vacuum concentration system: 1: Microwave oven, 2: Rotary, 3: Condenser tower, 4: Vacuum pump, 5: Vacuum regulator, 6: PLC-controller, 7: Cooling tank, 8: Temperature controller, 9: Cooling line](image)

MATERIALS AND METHODS

Preparation of syrup solution and pineapple

Syrup solution with 10 oBrix and the fresh pineapple juice sample (100 Litre) were obtained from Siam Agro Industry Co, Ltd. The fresh pineapple juice was then packed in plastic bag and kept at -20 °C for further experiment. Before the experiment the fresh pineapple juice was warmed until 30 °C.

Concentration methods

Two different evaporation processes with stirring rate of 50 rpm, were employed for production of syrup solution and pineapple juice concentrate. Syrup solution and pineapple
juice at 10 and 12 °Brix were placed into 1 liter evaporation flasks and evaporated until the concentration of 60.5 °Brix.

**Total soluble solids**
After evaporation, the concentrated pineapple juice was cooled to room temperature (20 ±1 °C) and measured soluble solids by refractometer.

**Color measurement**
After MVE and RVE, the juice samples were cooled to room temperature (20 ±1 °C). Color measurements of pineapple juice were carried out using Hunter lab colorimeter. Lightness ratio (L), redness (a) and yellowness (b) were recorded. Triplicate measurements were used for each determination. Total color difference (TCD) was calculated by using the following formula:

\[
TCD = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}
\]

Where, \(L_0, a_0\) and \(b_0\) represented the reading at time zero, and \(L, a\) and \(b\) represented the instantaneous individual readings during thermal treatment.

**Statistical Analysis**
One-way analysis of variance (ANOVA) was used in data analysis. Duncan multiple range test method was used to compare the differences of parameters differed at significance level of 0.05.

**Experimental design**
Syrup solution (500 mL) was warmed until reach 30 °C then evaporated to final 60.5 °Brix at three different power level (575, 428 and 321 watt) and three different vacuum degrees (-80, -70 and -50 kPa) by MVE. For comparison of evaporation methods on concentration rate and color change of pineapple juice (200 mL) at 12 °Brix was warmed until reach 30 °C then evaporated to a final 60.5 °Brix by MVE and RVE. During evaporation process, the change of pineapple juice samples were measured °Brix and \(L, a, b\) value

**RESULTS AND DISCUSSION**

**Effect of microwave power outputs and vacuum degrees on concentration change of syrup solution**
The syrup solution was concentrated to final 60.5 °Brix from initial 10 °Brix. The rate of syrup solution was increased exponentially for all power levels. (Maskan, 2004)

\[
C = C_0 + n \exp (kt)
\]

Where, \(C\) and \(C_0\) are the soluble solid concentration of pineapple concentrate at any time \(t\) and initial concentration, respectively; \(n\) is a constant and \(k\) is the evaporation rate constant (min\(^{-1}\)). Fig 2 shows the effect of three level of microwave power output at 575, 428 and 321.24 watts and three level of vacuum degree (-80, -70 and -50 kPa) on syrup solution concentrate rate. Table 1 shows evaporation rate of syrup solution from curve fitting method. For three different microwave power and vacuum conditions, the results showed that microwave power outputs were highly significant with the evaporation rates whereas the vacuum degrees showed slightly significant.
Table 1. Evaporation rate of syrup solution on effect of microwave power outputs and vacuum degrees.

<table>
<thead>
<tr>
<th>Power Level</th>
<th>-50 kPa</th>
<th>-70 kPa</th>
<th>-80 kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>575 Watts</td>
<td>13.45 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.41 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.24 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>428 Watts</td>
<td>9.89 ± 0.06</td>
<td>9.65 ± 0.06</td>
<td>9.54 ± 0.13</td>
</tr>
<tr>
<td>321 Watts</td>
<td>8.67 ± 0.1</td>
<td>8.30 ± 0.1</td>
<td>7.83 ± 0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean (n=3) ± Standard error of estimation.

Comparison on concentration rate of syrup solution and pineapple juice on quality change during evaporation processes

Concentration rate

Fig. 5 shows concentration pineapple juice from 12°Brix to the final concentration at 60.5°Brix. Concentration time was 34.5 and 85.5 min for MVE and RVE, respectively. The evaporation rate constant (k) for MVE was 2.5 times faster than RVE of concentrate pineapple juice. This is because of rapid heating and, hence evaporation water effect of microwave processing. (Zheng-Wei Cui et al., 2004)
Color Change

Fig 5 show that the changed color of pineapple juice was concentrated by two evaporation methods. The lightness ($L$) of concentrate pineapple juice decreased by 2.14 % and 3.18 % for $MVE$ and $RVE$ process. It indicated that samples become darker on both methods but the $MVE$ method gave lower $L$ value. The yellow pigment of concentrate pineapple juice were decreased by 1.77 % and 8.55 % for $MVE$ and $RVE$ when compare with fresh pineapple juice. The yellow pigment of concentrated pineapple juice from $MVE$ was better than $RVE$ because of its shorter time of concentration. Results from many researches were similar such as concentrated pomegranate juice (Maskan, 2004), pineapple juice during thermal process (Rattanathanalerk et al., 2004 and Chutintrasri and Noomhorm, 2005).

The redness ($a$ value) of pineapple juice of two evaporation method was increased by 7.0 % and 10.54 % for $MVE$ and $RVE$. $MVE$ provided less change in redness of pineapple juice because it took shorter time than $RVE$ in evaporation process. In conclusion, the total color difference (TCD) was increased by 0.25 and 2.23 % for $MVE$ and $RVE$ as consequences from $L$, $a$, $b$ value. This could be explained by the assumption that longer evaporation time effected the loss of yellowness (Chen et al., 1995), the acceleration of redness and becoming darker due to browning reaction.
CONCLUSION

According to the results obtained MVE could be used in evaporation process of syrup solution and pineapple juice concentrate successfully. MVE is a novel alternative methods of evaporation process of acceptable quality and gave better evaporation rate and saved more color than the traditional one.

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GOOD AGRICULTURAL PRACTICES FOR FRESH FRUIT AND VEGETABLES IN VIETNAM

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ABSTRACT
To overcome the food safety assurance disadvantages in its domestic and export markets, particularly since joining the WTO, Vietnamese Government has decided to drive the development of a national Vietnam GAP system; called VietGAP. VietGAP is based on the ASEAN GAP model (www.aphnet.org) but was also designed to meet the specific needs of the Vietnamese fresh fruit and vegetable industries. VietGAP consists of only one module but covering practices for all 4 components including food safety, environmental management, worker health, safety and welfare, and produce quality. The practices in VietGAP are aimed at preventing and minimizing hazards which would be occurred during food production chain including varieties, soil/media, fertilizers, chemicals, water and environmental impacts, worker health, safety and welfare. VietGAP will help Vietnamese growers, central markets and retailers to provide domestic and international customers and consumers with confidence in Vietnam’s fresh produce. A national VietGAP is expected to be officially released in early 2008 and would be the successful key for the Vietnamese fruit and vegetable industries.

Key words: good agricultural practice, quality assurance, food safety, fresh products

INTRODUCTION
In the horticulture industries Good Agricultural Practice (GAP) has become well established in the world, particularly in the European Union, USA, Chile, Australia, Japan and recently ASEAN. The Vietnamese horticultural industry is now facing more pressures from both domestic and international markets, particularly from the date joining the World Trade Organization (WTO) on 7th November 2006 because of its lack of a national GAP system.

Domestic
Nearly 23,000 Vietnamese were poisoned by foods including vegetable produce in the last four years (2001 – 2005) (Ministry of Health, 2006). Studies have found pesticide residues and other contaminants such as nitrates and heavy metals to be at levels well above maximum residue limits (MRL). In peri-urban agricultural areas such as Thanh Tri, Hoang Mai in Hanoi and Cu Chi, Hoc Mon in Hochiminh cities where the majority of vegetables are produced the soil contamination and environmental pollution are becoming severe due to over-uses of fertilizers and pesticides in agriculture and toxic waste – mainly heavy metals – poured from industrial companies. A large number of overseas funded projects from Australia (ACIAR, CARD) and Canada (CECI) have sought to introduce IPM and GAP to the Vietnamese horticultural industry. However Vietnam hasn’t at present established yet a national GAP system for horticultural produce.

International
Exporting vegetables and fruit to overseas, particularly to China which has been a traditional market for Vietnamese vegetables and fruit, has dramatically fallen from US$120.1 million in 2000 to US$24.9 million in 2004 (Table 1). In 2000, 57% of Vietnamese exported vegetables
and fruit went to China. In 2004 this fell to only 13%. Vietnamese experts believe this is a result of the Free-trade system of the Early Harvest Program of the China-ASEAN Free Trade Area, that China signed in 2003. China has substantially increased domestic production through major agricultural initiatives. Also, the preferences of Chinese consumers, particularly those in urban areas, have changed since China joined the WTO. Chinese consumers are seeking higher quality, clean and attractively packaged goods. Similar trends are occurring in the Vietnam domestic market as national wealth grows. China and other developing nations have responded to this need for assurances by accessing international standards and leveraging domestic governmental standards and control systems. Disaggregated Vietnamese horticulture industries have been slow to respond to these changing pressures and there is no national GAP system capability to drive and capture the improvements and assurances needed to compete in the new economic environment. Developing this capability is of greatly increased importance since Vietnam’s WTO accession on 7th November 2006.

Table 1. Value of vegetable & fruit exports from Vietnam to China and Total value 2000-2004.

<table>
<thead>
<tr>
<th>Year</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export to China, US$ million</td>
<td>120.5</td>
<td>142.0</td>
<td>121.5</td>
<td>67.1</td>
<td>24.9</td>
</tr>
<tr>
<td>(% total export to China)</td>
<td>(57%)</td>
<td>(43%)</td>
<td>(56%)</td>
<td>(37%)</td>
<td>(13%)</td>
</tr>
<tr>
<td>Total export, US$ million</td>
<td>213.1</td>
<td>329.9</td>
<td>218.5</td>
<td>182.5</td>
<td>186.8</td>
</tr>
</tbody>
</table>

The implementation of GAP programs currently within the ASEAN region varies, with some countries having government certified systems and others beginning the journey with awareness programs for growers(11). In 2004 Australian government was asked by the ASEAN Secretariat to help in establishing a GAP program for ASEAN. Under a project funded by the ASEAN Australia Development Cooperation Program (Quality Assurance Systems for ASEAN Fruit and Vegetables Project; No. 37703), a standard for ASEAN GAP was developed to harmonize GAP Programs in the region. The goal is to facilitate trade between ASEAN countries and to global markets, improve viability for farmers, and help sustain a safe food supply and the environment. ASEAN GAP was officially released on 22nd November 2006 in Indonesia (www.aphnet.org(1)). ASEAN GAP is an umbrella standard that individual ASEAN member countries will benchmark their national programs against to gain equivalence.

To overcome the disadvantages in terms of food safety in its domestic and export markets, it is time for Vietnamese Government to design and build the most suitable national VIETNAM GAP or VietGAP, based on ASEAN GAP, for the Vietnamese fresh fruit and vegetable industry to help growers, supermarkets and consumers. The Department of Plant Protection (DPP) and the Vietnamese Academy of Agricultural Sciences (VAAS) have been nominated by the Vietnamese Ministry of Agriculture and Rural Development (MARD) to develop a national GAP system for Vietnam.
**SCOPE OF VietGAP**

VietGAP consists of only one module but covering practices for all 4 components including food safety, environmental management, worker health, safety and welfare, and produce quality. The practices in VietGAP are aimed at preventing and minimizing hazards which would be occurred during food production chain including varieties, soil/media, fertilizers, chemicals, water and environmental impacts, worker health, safety and welfare. VietGAP has been divided into several sections from production, harvesting, and postharvest handling of fresh products on farm. It also covers postharvest handling in areas and/or sites where produce is washed and packed for sale. This enables progressive implementation of VietGAP on individual Vietnamese horticultural sector.

**DEVELOPMENT OF VietGAP**

To develop VietGAP, a series of meetings and workshops were held in Hanoi involving representatives from project teams of the Department of Plant Protection and Research Institutions of the Vietnamese Academy of Agricultural Science. The initial meetings focused on the translation of ASEAN GAP and experiences from implementing GAP programs in ASEAN (Malaysia, Thailand), EC and Australia. Certified systems and guidelines for GAP from EUREPGAP and Freshcare were also reviewed. Subsequent meetings refined the standard practices which originated from ASEAN GAP to ensure that the recommended practices in VietGAP were relevant and achievable for all Vietnamese fresh fruit and vegetable growers, but consistent with previous GAP practices which were mainly based on EUREPGAP. The followed workshops involved in thoroughly discussions between project teams and Grower Associations and Supermarkets, to make sure VietGAP is for Vietnamese fresh fruit and vegetable growers, but consistent with international GAP practices which were based on HACCP.

VietGAP consists of one module but covering practices for all 4 components including food safety, environmental management, worker health, safety and welfare, and produce quality. The practices in VietGAP have been divided into several sections including the selection of production area, variety, soil and media, fertilizers and soil additives, water uses, plant protection and chemicals, harvesting, postharvest, waste management, worker health, safety and welfare, recording and traceability, and internal audit of fresh fruit and vegetables on farm. Guidelines for implementing VietGAP for Vietnamese fresh fruit, vegetables and tea producers are currently being developed to enhance the understanding of what is required to implement the agricultural good practices in Vietnam. Plan of VietGAP training courses are also being developed for master trainers for all provinces in Vietnam.

A national VietGAP is expected to be officially released in early 2008 and would be the successful key for the Vietnamese fruit and vegetable industries.

**ACKNOWLEDGEMENTS**

The development of VietGAP is an activity within the projects; “Quy định chung về GAP trong sản xuất rau an toàn” (Department of Plant Protection, MARD) and “Guidelines for implementing VietGAP for Vietnamese fresh vegetables producers,” (Vietnamese Academy of Agricultural Science). Both projects belong to “Food safety” project initiated under and funded by the Ministry of Agriculture and Rural Development (MARD).

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1. Department of Plant Protection, MARD:
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FOOD STRUCTURE AND NOVEL PROCESSING TOOLS

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INTRODUCTION
Food structuring is a multidisciplinary approach underpinning the relevance of biochemistry, biophysics and material science for modern food processing. It shapes the properties of our foods and it is a powerful tool to control the interaction of nutrients and functional agents within the human body. Primarily, it is the design of interfaces by state-of-the-art and often unconventional technological means which can control the release of food components during digestion and hereby modulating the nutritional value, reducing toxicity or attenuating allergenity. In addition the distribution and dynamics of bulk and interfacing water is a major control point since reactivity of ingredients during production and storage of foods is directly correlated to the availability and the state of the water.

FOOD STRUCTURE
Interfaces formed by surfactant micelles, vesicles, bilayers, reverse micelles, and liquid crystals that allow a controlled release of polar, non-polar, and/or amphiphilic substances have been used for many years. An increasing market segment of food products that contain such delivery systems for specific tasks are often referred to as functional foods and are supposed to promote health or reduce the risk of disease beyond providing a purely nutritional product. Forced by specific legislations in US, EU or Japan, such claims on foods have to rely on scientific evidence that proves the case and which has to be provided from nutritional science. The challenge on the technological side is to develop and improve carriers, encapsulations and release systems of functional agents such as colors, flavors, antioxidants, nutrients and antimicrobials with advanced features and ready for use in conventional and novel food products.

Glycoproteins
Glycoproteins are highly important natural bioactive food components with enormous physiological functions, such as antibiotic properties. They possess protein bound sugar moieties, so-called glycans. Typical sources are egg proteins, e. g. ovalbumin, conalbumin, and ovomucoid, milk proteins, e. g. lactoferrin, κ-casein, and glycomacropeptide or immunoglobulins.

They contribute to the processing quality of foods, as stabilizers for emulsions, gels, and foams. Moreover, various glycoproteins are formed during processing of food products via interactions of carbohydrates and proteins.

Glycoprotein research is one of the emerging areas in food development with respect to biological and technological quality. A highly sensitive methodology is the CarboDeep technology of NutriCognia for the analysis of complex glycoprotein mixtures in food. The technology is based on a biochip arrayed with a series of well-characterized lectins. These lectins are able to recognize various glycan structures on the protein surface very specifically.

So far, several applications are available, next to the influence of thermal treatment we can identify ingredients such as lactoferrin in baby food, analyze the origin of milk (goat, sheep, buffalo, or cow) and the integrity of glycoproteins.
Multiple emulsions
Water-in-oil-in-water (W/O/W) multiple emulsions are emulsion systems where small water droplets are entrapped within larger oil droplets that in turn are dispersed in a continuous water phase. Because of the presence of a reservoir phase inside droplets of another phase that can be used to prolong release of active ingredients, multiple emulsions find many applications in industries such as pharmaceuticals and cosmetics. Multiple W/O/W emulsions contain both W/O and O/W simple emulsions and require at least 2 emulsifiers to be present in the system when prepared using the 2-step method, one that has a low Hydrophile-Lipophile Balance (HLB) value to stabilize the primary W/O emulsion and one that has a high HLB value to stabilize the secondary O/W emulsion.

The development of basics for multiple food emulsions of w/o/w-type opens a large innovation potential in the food industry. It will be possible to enlarge the water content of lipophilic phase without a negative influence on sensory properties and the energy content of food will be reduced. Substances, which should be not come into contact with environment because of stability or sensory reasons, can be included in the dispersed hydrophilic phase w1. Release properties of these substances can be controlled by melting properties of crystallized fat fractions in the lipophilic phase.

PROCESSING OPERATIONS
Apart from traditional processing routes, completely new and innovative approaches will have to be considered since the utilisation of nano-scale building blocks for structuring tasks requires substantial knowledge on quantum effects and mechanisms specific for extremely increased relative surface areas. Emphasis will be put on the generation of stable multiple emulsions, multilayer emulsions, biopolymeric nanoparticles and coatings. Based on biophysics of molecular assembly bio-mimetic structures will be exploited for highly selective separation problems.

Most food materials are complex dispersed systems and their structure determines quality characteristics, technical and physiological functionality as well as consumer acceptance. Modern product development includes design and modification of structure elements of the food systems with respect to matrix, interfaces and dispersed elements, i.e. gas bubbles, liquid droplets or solid particles. Profound knowledge is required to manage food structure formation on the molecular level to interface networks on the macroscopic scale by suitable technological means.

The efficiency of food processing operations primarily depends on the ratio between energy used for targeted action and stochastically dissipated energy. As foods are unstable from a thermodynamic point of view all kinds of reactions can potentially take place, which if uncontrolled may lead to the situation that the food becomes completely unfit for consumption. When heating foods, thermal energy is supplied to the food as a result of which the speed of reaction increases. A distinction can be made between chemical, biochemical, physical and microbiological reactions. Biochemical and microbiological reactions are essentially chemical reactions catalysed by enzymes. Physical reactions are not very temperature dependent. In contrast, biochemical and microbiological reactions are eliminated when a food is sufficiently heated due to inactivation of enzymes and microbes. Enzymes are inactivated because, as proteins, they are subject to heat denaturation, which results in the loss of their ability to catalyse reactions. The effect of heat on microbes is more complicated than just protein denaturation, and they are inactivated at elevated temperatures. Once biochemical and microbiological reactions have been eliminated, chemical reactions become the most important for consideration.
Without doubt, the competitiveness of food manufacturing companies is strongly related to their manufacturing procedures. Not only optimal energy usage, material efficiency and automation have a direct impact on the profit margins, but also aspects of safety and the drive for product innovation often demand novel processing techniques. Evaluating the suitability of the different means of energy transfer (thermal, physical, electrical, electro-magnetical, irradiation) or combinations of these is often the foundation for optimising processing technology.

**Pulsed electric fields processing (Elcrack®)**

Elcrack® is a highly efficient treatment to produce irreversible membrane breakdown of plant, animal or microbial cell membranes. Disintegration of biological cells often is a crucial pre-treatment step to improve mass transfer rates. A Elcrack® treatment can be utilized to substitute conventional disintegration techniques such as grinding, thermal or enzymatic treatments prior to processes such as drying, extraction or pressing. Pore induction by Elcrack® can, dependent on treatment intensity, also cause a loss of vitality and be utilized to achieve an inactivation of microorganisms in liquid media.

For improvement of mass transfer and for microbial inactivation treatment intensity has to be increased to achieve an irreversible membrane breakdown and a loss of its barrier function. The electric field strength has been identified as one main processing parameter, a cell specific threshold has to be exceed to induce a membrane pore. Further increasing the electric field strength has been found to enhance the efficiency of the treatment (Boyko et al., 1998; Heinz et al., 1999; Heinz and Knorr, 2000; McDonald et al., 2000).

Disintegration of cellular material, a key step prior to juice winning operations such as extraction or pressing is often performed by an enzymatic maceration, a thermal treatment or a mechanical grinding. These techniques may require a significant amount of thermal or mechanical energy as well as holding times and storage tanks for enzymatic maceration. Side activities of enzymes (added or natural) and thermal degradation during holding time can cause losses of nutritionally and physiologically valuable compounds and lower product quality. Applying PEF to cellular tissue an increase in mass transfer coefficients was observed due to cell membrane permeabilization. Based on this effect a PEF application can replace or substitute conventional techniques in fruit and vegetable juice processing.

**Food Quality Management**

In addition to the processing technology used, food safety is a crucial criteria in commercialising high-value products. Legislations and standards are becoming vital in this current climate of food-globalisation, where food companies are continually challenged to aim their products towards international markets. Effective food safety can only be guaranteed by involving all steps of the food chain in the safety management process. This gives the opportunity to retain the native quality of the agricultural raw materials and also encourages ‘minimal’ product processing and a reduction in the use of preservatives. Modern food production has to rely on quality management systems which adequately address the actual hazardous situation.

**Acrylamide**

The formation of Acrylamide by heat treatment is a good example for the importance of quality management systems. Since animal studies have shown that acrylamide is a genotoxic carcinogen, such foods with significant levels of acrylamide precursors within their matrices should be a matter of inspection. The formation of acrylamide in foods is a consequence of the Maillard reaction between asparagine and reducing sugars. Therefore, factors affecting the
concentration of these precursors in food, together with processing conditions during food preparation and storage will affect the final concentration of acrylamide in food.

Heated potato products are in the focus of public interest due to the high acrylamide contents detected in some of these products. Fried potatoes, e.g. French fries, potato wedges, hash browns, oven-fried potatoes and crisps are the most relevant products in this area, whereas boiled potatoes contain virtually no acrylamide. These high amounts are mainly caused by large contents of free asparagine as one of the main precursors in potato tubers. Those products are frequently monitored by government food authorities and a large amount of published data are available (see for example, [1] or, [2] for Germany or [3;4] for the USA). Maximum concentrations of 2310 µg/kg for ready-to-eat French fries and 4215 µg/kg for crisps were published by German food authorities for 2005 [5]. Median values are 212 µg/kg and 363 µg/kg, respectively, indicating a broad range of measured values in this kind of products. Heated potato products, e.g. French fries or chips, often are consumed as a part of daily diets, especially in the case of children or adolescent people. Therefore, these make a significant contribution to acrylamide intake [6]. According to Wilson et al. [7] acrylamide intake from fried potatoes and potato crisps represents 35% of total acrylamide intake in USA and up to 46% in the Netherlands. Biedermann-Brem et al. [8] estimated an additional intake of acrylamide of about 30 µg per day caused by one single portion of hash browns (250 g) per month. Due to the high amounts of acrylamide measured in potato products and their significant contribution to daily acrylamide intake, minimization efforts have to concentrate on these kind of products.

The reduction of any acrylamide formation relates to two main principles. One is a lowering of the precursor concentration in the raw material or in the prepared food to reduce the absolute potential. The other one relates to less heat treatment for a lower acrylamide value. Both approaches have to be considered carefully, because a reduction of acrylamide should maintain the overall organoleptic and nutritional quality of the products as far as possible. It could be demonstrated that frying under higher pressure (2 bars) leads to lower acrylamide contents in French fries for the same level of browning (quality). Therefore, pressure shifts the reaction processes during frying to lower acrylamide formation.

CONCLUSIONS

The processing of tailor-made food products with specific functions will require targeted function engineering which necessitate new processing tools as well as a re-evaluation of the potential of existing processing tools. To achieve better knowledge on process-, structure-property relationships new processing technologies will be required dealing with food products from the macro to the molecular scale. Such processes need to be robust and scale independent to allow their application at small and large scales. In addition a redesign and optimisation of food processing and packaging is necessary to increase commercial competitiveness and to create sustainable food processes. Among those new or redesigned process tools are those using mechanical forces (e.g. high pressure, extrusion, ultrasound), electrical fields (e.g. pulsed electric field processing), or electromagnetic fields (e.g. radio frequency heating).

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MICROENCAPSULATION OF LACTIC ACID BACTERIA ISOLATED FROM COMMERCIAL YOGURTS IN TAIWAN

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ABSTRACT

The calcium alginate beads containing microbial cells had a mean diameter of 2.56 ± 0.12 mm and 2.63 ± 0.15 mm (range 2.13-3.13 mm, using needle size of 1.20 mm) for L. delbrueckii subsp. lactis and S. salivarius subsp. thermophilus, respectively. Furthermore, the mean diameter of extruded microcapsules using needle size of 0.55 mm was 1.75 ± 0.13 mm for L. delbrueckii subsp. lactis and 1.84 ± 0.16 mm for S. salivarius subsp. thermophilus (range 1.38-2.13 mm). The morphology of the microcapsules was studied using a phase contrast scanning electron microscopy (SEM). Cross-sectional SEM revealed clusters of encapsulated bacteria in the porous alginate microspheres. L. delbrueckii subsp. lactis and S. salivarius subsp. thermophilus were tested for their tolerance to simulated conditions of the gastrointestinal tract at 37 °C. Resistance to simulated gastric juice (pH 2.0 and 3.0) varied among tested strains and needle sizes (0.55 mm and 1.20 mm) used for extrusion. The large encapsulated microspheres of both tested strains were significantly more resistant to low pH than the small one.

Key words: Microencapsulation, L. delbrueckii subsp. lactis, S. salivarius subsp. thermophilus

INTRODUCTION

Fermented dairy products, such as yogurt, have been widely accepted as vehicles of transmission of probiotics to consumers (Ouwehand and Salminen, 1998). Fermented milks are not only nutritious, but are also seen as therapeutic agents that enhance the general well-being of a person by exerting certain immunologic functions (Shah, 2000). For example, lactic acid bacteria (LAB) in yogurt, such as Lactobacillus bulgaricus and Streptococcus thermophilus, can improve lactose digestion for the lactose intolerant people (Kailasapathy and Rybka, 1997; Salminen and et al., 1998; Isolauri and et al., 2001; Saito, 2004)

Yogurt has been recognized as a health food because of its health properties such as improving digestion, boosting immunity and protection against carcinogen (Hamilton-Miller et al., 1999; De Roos and Katan, 2000; Ouyang et al., 2004). However, a yogurt product has limits about its shelf life and storage condition. Therefore, if we can improve and prolong the shelf life of yogurt product, the cost of transportation and storage can be reduced. Normally, yogurt can be kept at refrigerated temperature 4-6 °C for 30-40 days. On the other hand, LAB in yogurt, such as Lactobacillus bulgaricus and Streptococcus thermophilus, play a very important role in digestion system of humans. However, the LAB may not survive in sufficient numbers when incorporated into dairy products or during their passage through the gastrointestinal tract. Therefore providing probiotic living cells with a physical barrier against adverse environmental conditions is a necessity. Among the techniques available for immobilizing living cells, entrapment in calcium alginate beads has been frequently used for the immobilization of LAB. Alginate has the benefits of being non-toxic to the cells being
immobilized, and it is an accepted food additive (Sheu and Marshall, 1993; Gouin, 2004; Park et al., 2004; Chandramouli et al., 2004).

The objectives of this study were microencapsulation of isolated LAB in order to protect them in adverse conditions such as bile acid and salt in gastro-intestinal tract.

MATERIALS AND METHODS

Bacteria, growth conditions and preparation of cell suspensions

Pure cultures of bacteria *Lactobacillus delbrueckii* subsp. *lactis* and *Streptococcus salivarius* subsp. *thermophilus* were isolated from the commercial yogurt products in our previous research. Cells for survival experiments were propagated in 500 ml MRS broth for 24 hr at 37°C. Cells were harvested by centrifugation (Model CR21E, Hitachi, Japan) at 3000 × g, 4°C for 10 min, washed twice and re-suspended in saline to approximately 10^10 CFU/ml (Sheu and Marshall, 1993; Adhikari et al., 2003). Cell count was determined by anaerobic spread plate on MRS agar after 48 hr at 37°C.

The cell suspensions were subsequently used either directly (free cells) in assays or subjected to microencapsulation as described in next Section.

Procedure of microencapsulation of LAB

The capsules were prepared aseptically using a syringe with a needle. Different alginate capsule sizes in this experiment were achieved by using different sizes (0.55mm and 1.20mm) of sterile non-toxic needles (Terumo Corporation, Tokyo, Japan). The procedure of microencapsulation for *L. delbrueckii* subsp. *lactis* and *S. salivarius* subsp. *thermophilus* was shown as in Figure 1.

The cells suspension was dispersed in 1.5% (w/v) sterile sodium alginate solution thoroughly with the ratio of cells suspension to sodium alginate solution is approximately equal 1:4. The mixture was extruded through a needle (2) by a glass syringe (Hap Dong, Korea) (1). The droplets of cells and alginate fell into a beaker (4) of calcium chloride (CaCl₂) solution (0.1M), which was placed on magnetic stir plate (5), whereupon the alginate gels. The beads were kept in CaCl₂ solution for 30 min to strengthen the gel before rinsing them with sterile saline water (0.85% (w/v) NaCl). After hardening, the beads were filtered through a sterile plastic sieve.

Encapsulation parameters

The encapsulation parameters such as nozzle size (0.55 and 1.200 mm), 1.5% alginate concentration, calcium chloride concentration (0.1M), and hardening time of capsules in calcium chloride (30 min) were used for their efficacy in increasing the viability of encapsulated bacteria in simulated gastric conditions (Krasaekoopt et al., 2003; Chandramouli et al., 2004).
Examination of alginate beads

Diameters of calcium alginate beads, stained with safranine (Difco, Detroit), were measured with an eyepiece micrometer on an optical microscope at a magnification of 40\(\times\) (Microscope model YS2-T, Nikon, Japan). At least 140 randomly selected beads were measured for each sample (Sheu and Marshall, 1993). For the bigger sizes of calcium alginate beads, the measurement was taken by using the Mitutoyo micrometer (Japan).

A scanning electron microscopy (SEM) was employed to examine the external and internal appearance of calcium alginate beads. The calcium alginate beads were subjected to alcohol dehydration which was described as following statements:

Calcium alginate beads (specimen) were placed in fixative (2.5% glutaraldehyde buffered with 0.1 M PO\(_4\) buffer, pH 7.2) for 4 hr (2 hr each at 4 °C and room temperature). Discard the fixative and rinse twice in the buffer (0.1 M PO\(_4\) buffer) for 15 min per each time, and then the specimen was dehydrated in ascending ethanol concentrations (10%, 25%, 50%, 75%, 95%, and 100%) for 10 min per each ethanol concentration. In ethanol 100%, the hydrate action was repeated 3 times. The last step of dehydration was using acetone for 10 min, and keeps the sample in acetone in a tightly capped vial (using Durham test tube) until ready to

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**Figure 1. A schematic microencapsulated experimental set-up.**

(1) - Syringe
(2) - Needle
(3) - Alginate gels
(4) - Beaker
(5) - Magnetic stir plate
(6) - Speed control knob
(7) - Heat control knob
perform the drying procedure. After dehydration, specimens were dried at the critical-point by using Hitachi Critical Point dryer (Model HCP-2, Hitachi Koki Co., Ltd., Tokyo, Japan). In order to observe internal appearance, alginate microspheres were fractured with a razor blade.

The dried specimens were mounted on a two-sided adhesive carbon tape, which were pasted on an aluminum stub, and coated with 10 nm gold for 180 seconds by using E1010 ion Sputter (Hitachi Science Systems, Ltd., Japan). Microscopy was performed on a Scanning Electron Microscope (Model S-3000N, Hitachi, Japan) at an accelerating voltage of 15 kV. Images were captured as grayscale digital images in TIFF format (Sheu and Marshall, 1993; Muthukumarasamy et al., 2006).

**Efficacy of cell release from capsules**

To determine the viable counts of the entrapped bacteria, 0.1 g of capsules were re-suspended in 10 ml of phosphate buffer (0.1 M, pH 7.0) followed by gentle shaking at room temperature (75 rpm). Samples were taken at different time intervals to determine the complete release of encapsulated bacteria by plating on MRS agar (Chandramouli et al., 2004).

**Survival of free and encapsulated bacteria in simulated gastric conditions**

The encapsulated and free cells of LAB were tested in simulated gastric juice (SGJ) (0.08 M HCl containing 0.2% NaCl) at different pH values (pH2, pH3, and pH7 as control) for survival of LAB over 2 hr at 30-min intervals (Hansen et al., 2002; Muthukumarasamy et al., 2006). To study the effects of microencapsulation on LAB survival in SGJ, 1 ml (measured by displacement of water) of microcapsules was added in triplicate to 9 ml of the simulated gastric juice pre-warmed to 37 °C, and viability of LAB was followed as noted above. Capsules were harvested every 30 min by poured SGJ out of the test tube, added to 9 ml of 0.1 M phosphate buffer, and ground using a sterile glass rod. Serial dilutions were prepared in saline and plated on MRS-CaCO3 agar. Plates were incubated anaerobically for 48 hr at 37 °C. Experiments were done in duplicate.

**Survival of LAB in simulated bile juice**

The resistance to bile salts was determined by inoculating free and encapsulated cells in simulated bile juice (SBJ) made with MRS broth pH 6.9 containing 0 (control), and 12.0 g/l bile salts (Sigma-Aldrich Inc., USA), pre-warmed at 37 °C (Hansen et al., 2002; Muthukumarasamy et al., 2006). Triplicate samples were withdrawn after incubation at 37°C for 0, 3 and 6 hr and cell counts of free and encapsulated bacteria were enumerated on MRS agar after anaerobic incubation at 37°C for 48 hr.

**Statistical analysis**

The mean values and the standard deviation were calculated from the data obtained from three separate experiments. These data were then compared by the Duncan’s multiple range test using Statistical Analysis System (SAS) for windows version 9.1 (SAS institute Inc., North Carolina, USA, 2003).

**RESULTS AND DISCUSSION**

**Microcapsule morphology**

Figure 2 shows the scanning electron microscopy (SEM) of external alginate beads of *L. delbrueckii* subsp. *lactis* (Figures 2a and 2b) and *S. salivarius* subsp. *thermophilus* (Figures 2c and 2d). The surfaces of microcapsules of two strains (*L. delbrueckii* subsp. *lactis* and *S. salivarius* subsp. *thermophilus*) were smoother when using needle size of 1.20 mm than using needle size of 0.55 mm. Moreover, SEM revealed that the microencapsulation methods used
here produced a consistent mixture of spherical alginate microspheres with a smooth surface (Figures 2b and 2d) and a rough surface (Figures 2a and 2c).

Micrographs of fractured alginate microspheres revealed encapsulated bacteria in small voids, both alone or in groups, which were distributed throughout the alginate network (Figure 3). Clusters of \textit{S. salivarius} subsp. \textit{thermophilus} in porous alginate microsphere illustrated very clearly when they were observed under SEM at different magnifications (Figure 4).

Figure 2. Scanning electron microscopy of the whole alginate microspheres. (a) and (b) were \textit{L. delbrueckii} subsp. \textit{lactis} alginate beads extruded through needle sizes of 0.55mm and 1.2mm, respectively; (c) and (d) Microcapsules of \textit{S. salivarius} subsp. \textit{thermophilus} extruded through needle sizes of 0.55mm and 1.2mm, respectively.
Figure 3. SEM of the cross-sectional morphology of LAB-alginate beads. (a) and (b) were *L. delbrueckii* subsp. *lactis* alginate beads extruded through needle size 0.55mm and 1.2mm, respectively; (c) and (d) Microcapsules of *S. salivarius* subsp. *thermophilus* extruded through needle size 0.55mm and 1.2mm, respectively.

Figure 4. SEM of microsphere’s (needle size of 0.55 mm) fracture of *S. salivarius* subsp. *thermophilus* at different magnifications. (a) at 450×, (b) at 3000×, and (c) at 10000×.
Diameter of microcapsules containing LAB

Figure 5 shows the calcium alginate beads containing microbial cells with mean diameters of 2.56 ± 0.12 mm and 2.63 ± 0.15 mm (bead sizes in gap of 2.13-3.13 mm, using needle size of 1.20 mm) for \textit{L. delbrueckii} subsp. \textit{lactis} and \textit{S. salivarius} subsp. \textit{thermophilus}, respectively. In addition, the mean diameter of extruded microcapsules using needle size of 0.55 mm was 1.75 ± 0.13 mm for \textit{L. delbrueckii} subsp. \textit{lactis} and 1.84 ± 0.16 mm for \textit{S. salivarius} subsp. \textit{thermophilus} (bead sizes interval of 1.38-2.13 mm).

Efficacy of cells release from capsules

The results of this study show that there was a complete release of microencapsulated \textit{L. delbrueckii} subsp. \textit{lactis} in 0.1 M phosphate solution within 30 min with gentle shaking (Figure 6). Under the similar conditions, the release of microencapsulated \textit{S. salivarius} subsp. \textit{thermophilus} took only about 15 min (Figure 7).

![Figure 5](image-url)

Figure 5. Size range of small and large alginate microspheres loaded with LAB. The mean diameter curves of \textit{S. salivarius} subsp. \textit{thermophilus} and \textit{L. delbrueckii} subsp. \textit{lactis} extruded through needle size of 0.55 mm and that of \textit{S. salivarius} subsp. \textit{thermophilus} and \textit{L. delbrueckii} subsp. \textit{lactis} extruded through needle size of 1.20 mm.
Figure 6. Efficacy of encapsulated *L. delbrueckii* subsp. *lactis* release from the capsules. The needle sizes used were 0.55 and 1.20 mm.

Figure 7. Efficacy of encapsulated *S. salivarius* subsp. *thermophilus* release from the capsules. The needle sizes used were 0.55 and 1.20 mm.
Survival of L. delbrueckii subsp. lactis in simulated gastric juice (SGJ)

In SGJ at pH 2, free cells of L. delbrueckii subsp. lactis did not survive after 90 min of incubation at 37 °C when the initial level of viable cell count was at 8.12 ± 0.07 log CFU/ml. Our result was the same as that reported by Fernández et al. (2005). The survival of different Lactobacilli in acidic conditions varied. The survival rate of Lactobacillus paracasei NFBCC338 declined to undetectable levels after only 30 min of exposure (Corcoran et al., 2005). Table 1 shows the ability of encapsulated L. delbrueckii subsp. lactis to survive exposure in different pH of SGJ. At initial loaded numbers 7.06 – 7.23 (log CFU/ml) for encapsulated L. delbrueckii subsp. lactis, which was extruded through a needle size of 0.55 mm, in SGJ with different pH of 2, 3, and 7 (control), there was a decrease of around 2 log CFU/ml (pH 3 and pH 7) and 3 log CFU/ml (pH 2) after an hour of incubation at 37 °C. Moreover, there was no viable cell detected in SGJ at pH 2 after 2 hr of incubation at 37 °C. The limit of detection was less than 1 log CFU/ml. The reason for this decrease might be that the wall matrix of calcium alginate gel could not protect the planktonic cells in low pH for long time (2 hr). The encapsulated L. delbrueckii subsp. lactis survived well in SGJ at pH 3, although there was a significant difference (P < 0.05) in the number of survival cells in SGJ between pH 3 and pH 7. On the other hand, the results show that the number of cells declined around 3 log CFU/ml in SGJ at pH 3 and pH 7 (control). The reason could be attributed to the release of viable cells during incubation in SGJ. In addition, the survival cells were counted only from the capsules harvested by filtration.

The results in this study show that larger microcapsules (2.56 ± 0.12 mm) were better able to protect L. delbrueckii subsp. lactis than smaller microcapsules (1.75 ± 0.13 mm) (Table 1). There were 2.56 ± 0.11 log CFU/ml survived in SGJ at pH 2 after 2 hr incubation at 37 °C. Lee and Heo (2000) reported that large alginate capsules (2.63 mm) offered more protection to Bifidobacterium longum cells against acid challenge than smaller capsules (1.03 mm). Microencapsulation in alginate microspheres with mean diameters of 20 and 70 μm did not significantly improve survival of bifidobacteria during exposure to SGJ (Hansen et al., 2002). Other work of Muthukumarasamy et al. (2006) has shown that the bigger (2 to 4 mm) extruded capsules protected the cells better than the smaller (20 μm to 1 mm) emulsified capsules. They explained that the larger size of these capsules may have afforded additional physical protection simply by increasing the distance between encapsulated cells and the acid.

Survival of S. salivarius subsp. thermophilus in SGJ

Table 2 shows the viability of encapsulated S. salivarius subsp. thermophilus in SGJ during challenge at low pH, incubation at 37 °C for 2 hr.

As been stated earlier on L. delbrueckii subsp. lactis, viable free cells of S. salivarius subsp. thermophilus were eliminated after 90 min, when it was subjected to SGJ challenge at pH 2 and the number of cells loading was 8.07 ± 0.04 log CFU/ml. The results show that the survival of encapsulated S. salivarius subsp. thermophilus in SGJ at pH 3 was significantly (P < 0.001) better than the survival rate at pH 2 for encapsulated S. salivarius subsp. thermophilus with both small (1.84 ± 0.16 mm) and large (2.63 ± 0.15 mm) microsphere sizes. At pH 3 and pH 7, encapsulated S. salivarius subsp. thermophilus was reduced by less than 1 log CFU/ml in SGJ after 2 hr incubation at 37 °C, while at pH 2 there was a loss of around 6.5 log CFU/ml (Table 2).

Tables 1 and 2 show that the strain S. salivarius subsp. thermophilus was more sensitive to high acid as compared to L. delbrueckii subsp. lactis at pH 2 and with small microspheres. The number of cells survived of encapsulated L. delbrueckii subsp. lactis was 4.05 ± 0.81 log
CFU/ml, while the number of cells survived of encapsulated *S. salivarius* subsp. *thermophilus* was 1.78 ± 0.25 log CFU/ml after 1 hr incubation.

**Table 1. Number of viable cells (Mean ± SD) of microencapsulated *L. delbrueckii* subsp. *lactis* in SGJ at 37 °C**

<table>
<thead>
<tr>
<th>pH</th>
<th>Time of incubation (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>L05 (log CFU/ml)</td>
<td>2.0</td>
<td>7.19 ± 0.16</td>
<td>5.62 ± 0.07</td>
<td>4.05 ± 0.81</td>
<td>nd¹</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>7.23 ± 0.07</td>
<td>5.13 ± 0.02</td>
<td>5.28 ± 0.40</td>
<td>4.52 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>7.06 ± 0.08</td>
<td>5.16 ± 0.02</td>
<td>4.95 ± 0.07</td>
<td>4.24 ± 0.34</td>
</tr>
<tr>
<td>L12 (log CFU/ml)</td>
<td>2.0</td>
<td>7.51 ± 0.05</td>
<td>6.03 ± 0.26</td>
<td>5.40 ± 0.51</td>
<td>2.56 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>7.38 ± 0.05</td>
<td>6.61 ± 0.43</td>
<td>5.69 ± 0.12</td>
<td>5.83 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>7.49 ± 0.04</td>
<td>6.38 ± 0.17</td>
<td>5.04 ± 0.06</td>
<td>5.33 ± 0.04</td>
</tr>
</tbody>
</table>

¹Value was not detected (lowest limit of detection was ≤ 1 log CFU/ml). L05 and L12 are encapsulated *L. delbrueckii* subsp. *lactis* extruded through the needle sizes of 0.55 mm and 1.20 mm, respectively.

**Table 2. Number of viable cells (Mean ± SD) of microencapsulated *S. salivarius* subsp. *thermophilus* in SGJ at 37 °C**

<table>
<thead>
<tr>
<th>pH</th>
<th>Time of incubation (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>S05 (log CFU/ml)</td>
<td>2.0</td>
<td>8.54 ± 0.09</td>
<td>5.68 ± 0.11</td>
<td>1.78 ± 0.25</td>
<td>nd¹</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>8.13 ± 0.25</td>
<td>7.68 ± 0.14</td>
<td>7.59 ± 0.09</td>
<td>7.54 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>8.25 ± 0.10</td>
<td>7.98 ± 0.10</td>
<td>7.85 ± 0.09</td>
<td>7.42 ± 0.12</td>
</tr>
<tr>
<td>S12 (log CFU/ml)</td>
<td>2.0</td>
<td>8.21 ± 0.13</td>
<td>6.83 ± 0.05</td>
<td>4.95 ± 0.06</td>
<td>1.60 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>8.32 ± 0.16</td>
<td>7.70 ± 0.18</td>
<td>7.66 ± 0.28</td>
<td>7.59 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>8.13 ± 0.24</td>
<td>7.93 ± 0.02</td>
<td>7.91 ± 0.06</td>
<td>7.72 ± 0.07</td>
</tr>
</tbody>
</table>

¹Value was not detected (lowest limit of detection was ≤ 1 log CFU/ml). S05 and S12 are encapsulated *S. salivarius* subsp. *thermophilus* extruded through the needle sizes of 0.55 mm and 1.20 mm, respectively.

**Survival of LAB in simulated bile juice**

At the initial free cells load of 8.27 ± 0.04 log CFU/ml and 8.31 ± 0.06 log CFU/ml for *L. delbrueckii* subsp. *lactis* and *S. salivarius* subsp. *thermophilus*, respectively, both strains of *L. delbrueckii* subsp. *lactis* and *S. salivarius* subsp. *thermophilus* did not survive in bile juice (1.2% bile salt) for 3 hr (detected limit < 4 log CFU/ml) and 6 hr (detected limit < 3 log CFU/ml).

The results in this study show that microencapsulation method did not improve the survival of both *L. delbrueckii* subsp. *lactis* and *S. salivarius* subsp. *thermophilus*, except for encapsulated *L. delbrueckii* subsp. *lactis* extruded through the needle size of 1.20 mm. There was a decline of around 4 log CFU/ml of encapsulated *L. delbrueckii* subsp. *lactis* (extruded through needle size of 1.20 mm) after it was subjected to bile salt solution (1.2%) for 6 hr (from 9.27 ± 0.18 log CFU/ml to 5.58 ± 0.06 log CFU/ml).
CONCLUSION
Based on the findings of this study, we conclude that microencapsulation of the tested strains of bacteria improved the number of viable cell count when exposure at low pH conditions.

REFERENCES


Session 3
ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA (LAB) OF THE ‘NEM CHUA’ – FERMENTED MEAT PRODUCT OF VIETNAM

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ABSTRACT

Nem Chua is a fermented meat product that is very popular in Vietnam. It is made of ground lean pork mixed with boiled swine skin cut into thin strings, then the meat paste is formed in cubes (2cm x 3cm x 3cm) on which some producers place a thin slice of garlic and/or chili for decoration and flavor. The cubes are wrapped firstly by a leaf of special trees, next a plastic film, and lastly banana leaves. The special packaging, on the one hand, contributes to the initial microbial flora of Nem Chua and, on the other hand, creates favorable conditions for the Nem Chua’s fermentation during 3 – 4 days at ambient temperature. The fermentation takes place either spontaneously, or is orientated by adding a small percentage of fermented meat paste.

The aim of our study is to further understand the lactic microbial flora in order to select the most appropriate isolates used as starters of this fermented meat product.

Firstly, the actual manufacture of Nem Chua in four typical producers of three southern provinces was investigated, giving the overview on the environmental factors influencing the growth of the lactic acid bacteria in the product. The change of pH and LAB population during Nem Chua’s fermentation were examined. The percentage of LAB colony types appearing on MRS agar was counted, and finally, API 50 CH strips with the API 50 medium of BioMérieux were used to identify the colonies.

The fresh lean pork taken from the processing units had the initial pH of 5.61 ± 0.02, then pH decreased to 4.20 ± 0.11 in spontaneous fermentation, or 4.00 ± 0.20 in orientated fermentation at day 5th of fermentation. The final difference between the two types of fermentation was not significant; however, pH kinetics significantly differed (p<0.05). LAB increased for the first four days, reaching 2x10^8 CFU/g meat paste of spontaneous fermentation and 1.5x10^8 CFU/g meat paste of orientated fermentation. A slight reduction was afterwards noticed. On the MRS medium, there were 5 types of colonies at the beginning and only to 1 or 2 types at the end of fermentation. Among 131 isolates identified from Nem Chua, the proportions were 25.19% Lb. brevis, 21.37% Lb. plantarum, 14.50% Leuconostoc mesenteroides dextranicum, 12.21% Pediococcus pentosaceus and 11.45% Lactococcus lactis. The other LABs such as Lb. paracasei ssp paracasei, Lb. fermentum, Lb. cellobiosus, Lb. collinoides, Leuconostoc lactis existed in low percentage (from 1.53% to 3.82%).

INTRODUCTION

Nem Chua is a fermented meat product that is very popular in Vietnam. It is made of ground lean pork, mixed with boiled swine pig cut into thin strings, then the meat paste is formed in cubes (2cm x 3cm x 3cm) on which some producers place a thin slice of garlic and/or chili for decoration and flavor. The cubes are wrapped firstly in a leaf (commonly, this can be of ‘Chum ruot’ – Phyllanthus acidus, ‘Vong’ – Erythrina variegata or ‘Oi’ – Psidium guajava depending on the region), next a plastic film, and lastly banana leaves. The special packaging, on the one hand, contributes to the initial microbial flora of Nem Chua and, on the other hand,
creates favorable conditions for the Nem Chua’s fermentation during 3 – 4 days at ambient temperature. The fermentation takes place either spontaneously in the traditional production, or orientated by adding a small percentage of fermented meat paste in other processing units.

In fact, the products are unstable and must be quickly used after fermentation if they are put in the ambient temperature, which is the traditional mode of use in the country, or within month if they are kept in cold. Moreover, the Vietnamese processors often meet technological and qualitative problems because of defects related with undesirable microorganisms. The application of starters imported and commercialized with the intention of preventing the harmful flora for fermentation does not satisfy the sensory request of the Vietnamese consumers. The utilization of starters composed of microorganisms isolated from the autochthonous products seems to be the most reasonable choice.

The aim of our study is to further understand Nem Chua’s LAB in order to select among them the most appropriate isolates used as starters for this fermented meat product.

MATERIALS AND METHODS
A study was performed from March 2006 till March 2007 at Faculty of Food Sciences and Technologies - NÔNG LÂM University, Ho Chi Minh City, Vietnam, and at Unit of the Microbiological Security of Food (USMA) – University Bordeaux 1 of France.

Mediums and equipments
- MRS agar (Merk, pH = 5.5 ± 0.2) for counting LAB population, MRS agar and broth (Difco, pH = 6.5 ± 0.2) for LAB isolation and preservation, MRD – Maximum Recovery Diluent (Difco)
- pH-meter (Metrohm model 744, Switzerland and Mettler – Toledo GmbH, Switzerland)
- API 50 CH strips used along with API 50 CH L medium (BioMérieux) to identify the lactic acid bacteria

Methodology
Sampling and preparation of samples
Three samplings were taken from each of four typical processing units in three southern provinces, Vietnam (Binh Dinh, Ho Chi Minh City and Dong Thap). The processing units chosen were representatives for two ways of fermentation, including spontaneous fermentation (2 units) and oriented fermentation (2 units). In each sampling, 3 types of samples were chosen including lean pork (RM), paste meat (PM) and Nem Chua that had just made (not fermented, called N0). Three samples of each types were preserved in icebox. In addition, other samples of N0 were kept at ambient temperature to allow fermentation for 1, 2, 3, 4 and 5 days (called N1, N2, N3, N4 and N5, respectively; 3 samples for each tested time of fermentation) as that in the processing procedure of the units. All samples were transported immediately to laboratory to analyze at the determined time.

Each sample (approximately 10g) was added the diluent MRD (Difco) at a proportion 1:9 and homogenized in stomacher. Then, serial dilutions up to 10-6 were used for enumeration of the LAB population on MRS agar (Merk) according to the standard ISO 15214-1998. Each suspected LAB colony was purified twice by streaking on MRS agar (Difco) at 30°C for 48h. The pure cultures were grown in MRS broth (Difco) at 30°C for 48hours, afterward added glycerol at the rate of 10% and stored as stock cultures at -70°C.
Morphological, physiological and biochemical tests
On every Petri dish enumerated, the form and the dimension of colonies were initially observed, then the percentage of all typical forms of colonies was noticed, and finally, one colony representing for each type of existing colonies was chosen to study the morphological, physiological and biochemical characters of the bacteria.

Identification of the lactic bacteria
Among the LAB strains isolated, 131 strains were chosen of which the number of colonies was proportional with their percentage of appearance during five days of fermentation. The strains were selected after checking their purity on MRS agar incubated at 37°C for 48 hours. Each microtubes of API 50 CH strips was added the suspension API 50 CHL medium, and inoculated with pure colonies at an opacity of 2 on the scale of McFarland’s solutions. The results of fermentation of 49 carbohydrates after incubation at 37°C during 48 hours were considered following the document provided by BioMérieux.

2.3. Statistical analysis
Statistical evaluations were carried out by Fisher test with the software Minitab 12.21 to determine significant differences between samples and processing units.

RESULTS AND DISCUSSION
Change of pH during Nem Chua fermentation
pH changes in raw meat, paste meat and Nem Chua during the first five days of the fermentation are presented in Table 3.1.

Table 3.1 pH of raw meat, paste meat and Nem Chua during Nem Chua’s fermentation

<table>
<thead>
<tr>
<th>Fermentation Types</th>
<th>Processing units</th>
<th>Statistical item</th>
<th>RM</th>
<th>PM</th>
<th>N0</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>Calculation for processing units</th>
<th>Calculation for fermentation types</th>
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<tbody>
<tr>
<td>Út Thăng</td>
<td></td>
<td></td>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>64</td>
<td>n = 128</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>5.62</td>
<td>5.95</td>
<td>6.33</td>
<td>4.56</td>
<td>4.16</td>
<td>4.05</td>
<td>3.99</td>
<td>3.94</td>
<td>4.82°</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
<td>0.03</td>
<td>0.06</td>
<td>0.03</td>
<td>0.15</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
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</tr>
<tr>
<td>Giáo Thương</td>
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<td>n</td>
<td>8</td>
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<td>8</td>
<td>8</td>
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<td>X = 4.87°</td>
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<td>4.01</td>
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<td></td>
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<td>0.06</td>
<td>0.04</td>
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<td>0.12</td>
<td>0.03</td>
<td>0.01</td>
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<td>5.94</td>
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<td>4.12</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
<td>0.11</td>
<td>0.10</td>
<td>0.09</td>
<td>0.06</td>
<td>0.15</td>
<td>0.22</td>
<td>0.09</td>
<td>0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Bình Định</td>
<td></td>
<td></td>
<td>n</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>6</td>
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<td>8</td>
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<td>X = 5.85°</td>
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<tr>
<td></td>
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<td>0.03</td>
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<td>0.13</td>
<td>0.22</td>
<td>0.09</td>
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<td>0.10</td>
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<td>Calculation for samples</td>
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<td></td>
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<td>X</td>
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<td>5.85°</td>
<td>6.14°</td>
<td>5.26°</td>
<td>4.50°</td>
<td>4.24°</td>
<td>4.16°</td>
<td>4.08°</td>
<td>4.08°</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
<td>0.10</td>
<td>0.13</td>
<td>0.29</td>
<td>0.56</td>
<td>0.38</td>
<td>0.24</td>
<td>0.18</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

(n = Number of samplings; SD = Standard Deviation)
There was a significant difference between pHs of samples from four processing units at different time of fermentation (p < 0.05). The variance analysis for pH values measured during the Nem Chua’s fabrication showed that the pH differences for the first four days was significant (p<0.05). Nevertheless, the values was not different between the last two days of the Nem Chua’s fermentation (p>0.05).

pH of lean meat used at the four studied producers insignificantly differed (p > 0.05). The pH kinetics significantly differed between the orientated fermentation and the spontaneous one; however, for each type of fermentation, no significant difference was detected between two representative processing units (p > 0.05). In samples of spontaneous fermentation, no difference between pH of the lean pork and the Nem Chua which had just manufactured (N0) while that was contrast in the samples of orientated fermentation. That should be due to the addition of certain percentage of the fermented meat paste in the receipt. pH decreased more quickly as of the first day and then this evolution was stable towards the third day of orientated fermentation while the pH reduction significantly remarked only after 48 hours and afterward stable from the fourth day of spontaneous fermentation (p <0.05).

Over years, an empirical addition of lactic acid bacteria traced an efficacious antagonistic effect against the undesirable flora of the product. The utilization of classical starters that initiate rapid acidification of the raw meat aims to prevent the growth of a spoilage flora and thus, to extend shelf life, to reduce the production costs and moreover, to improve the quality of final products. Recently, the use of new, functional starters with an industrially or nutritionally important functionality is being explored. Functional starters offer an additional functionality compared to classical starters and represent a way of improving and optimizing the sausage fermentation process and achieving tastier, safer, and healthier products (Ammor et al., 2005; Leroy et al., 2005).

Quantitative changes of LAB during Nem Chua’s fermentation

Results of LAB count in raw meat and Nem Chua’s during the first five days of the fermentation are presented in Table 3.2.

The analysis of variance for LAB number measured during Nem Chua’s fabrication shows the significant difference between time examined (p<0.05). LAB population in raw meat used at the processing unit Binh Dinh quantitatively differed from others. On the other hand, there was not significant difference between raw meat and Nem Chua of day 0 (just wrapped and not fermented) except for those from the processing unit Giao Tho. That should be due to adding more amount of fermented meat in the receipt of this producer in comparison to the second producer of the orientated fermentation type, i.e. adding 10kg paste meat of the previous day and 80g Nem Chua of 24 hours fermentation to 40kg paste meat at the processing unit Giao Tho versus 500g of Nem Chua of 4 days fermentation for 30 kg lean meat at other processing units).

The kinetics of LAB number did not differ between two fermentation types (p>0.05). In general, the lactic population continued to increase during three first days and then decreased in the following days. For the first 24 hours of fermentation, the growth of lactic population in the orientated fermentation was more important than that of spontaneous way (a difference of 10^7 CFU/g). Nevertheless, the speed of growth in spontaneously fermented samples was more remarkable than that of oriented ones in 24 hours later (an increase by 1.16 decimal logarithmic value in the spontaneous fermentation vs 0.92 decimal logarithmic value in the orientated ones). The dynamic of LAB population was stable after three days of fermentation at four processing units (p> 0.05).
### Table 3.2 LAB numbers in raw meat and Nem Chua during fermentation (log_{10} CFU/g)

<table>
<thead>
<tr>
<th>Fermentation Types</th>
<th>Processing units</th>
<th>Statistical item</th>
<th>RM</th>
<th>PM</th>
<th>N0</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>Calculation for processing units</th>
<th>Calculation for fermentation types</th>
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<td></td>
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<td>3</td>
<td>3</td>
<td>3</td>
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</tr>
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<td>Út Thăng</td>
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</tr>
<tr>
<td></td>
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<td>X</td>
<td>5.43</td>
<td>5.52</td>
<td>5.53</td>
<td>7.23</td>
<td>7.92</td>
<td>8.94</td>
<td>7.74</td>
<td>7.34</td>
<td>6.96&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td></td>
<td>SD</td>
<td>0.16</td>
<td>0.12</td>
<td>0.06</td>
<td>0.33</td>
<td>0.02</td>
<td>0.14</td>
<td>0.09</td>
<td>0.11</td>
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<td>3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>5.27</td>
<td>5.3</td>
<td>6.38</td>
<td>7.33</td>
<td>8.48</td>
<td>8.66</td>
<td>8.02</td>
<td>7.68</td>
<td>7.14&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td>SD</td>
<td>0.19</td>
<td>0.2</td>
<td>0.04</td>
<td>0.1</td>
<td>0.01</td>
<td>0.06</td>
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<td>0.05</td>
<td>1.29</td>
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<td>Thử Đức</td>
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<tr>
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<td></td>
<td>X</td>
<td>4.76</td>
<td>5.5</td>
<td>5.63</td>
<td>6.97</td>
<td>8.4</td>
<td>8.38</td>
<td>8.15</td>
<td>7.95</td>
<td>6.97&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>SD</td>
<td>0.18</td>
<td>0.06</td>
<td>0.31</td>
<td>0.11</td>
<td>0.02</td>
<td>0.06</td>
<td>0.03</td>
<td>0.06</td>
<td>1.31</td>
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<td>3</td>
<td>24</td>
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<td>X</td>
<td>5.97</td>
<td>6.08</td>
<td>5.86</td>
<td>7.04</td>
<td>7.92</td>
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<td></td>
<td></td>
<td>SD</td>
<td>0.12</td>
<td>0.07</td>
<td>0.04</td>
<td>0.26</td>
<td>0.34</td>
<td>0.22</td>
<td>0.49</td>
<td>0.03</td>
<td>1.23</td>
</tr>
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<td>12</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Calculation for samples</td>
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<td></td>
<td>X</td>
<td>5.36&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.60&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.85&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.84&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>0.51</td>
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<td>0.24</td>
<td>0.31</td>
<td>0.26</td>
<td>0.39</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

(n = Number of sampling; SD = Standard Deviation)

A compatibility was observed between the kinetics of pH, and those of LAB population, in which the coefficient of exponential regression equations (R^2) were very strong (76.9% - 91.4% at p<0.05). Bourgeois and Larpent (1989) noted the growth of *Lactobacillus* was favorable at pH from 6.4 to 4.5 but more limited at pH 4.0-3.6. The LAB population was reduced for two last days observed would be due to the unfavorable pH of the medium at the moments of observation. For dry sausages, *Lactobacillus* initially presented at 10<sup>3</sup> – 10<sup>4</sup> CFU/g and attained 10<sup>6</sup> – 10<sup>8</sup>CFU/g at the end of curing in this French traditional product (Fournaud et al., 1976).

**Dynamics in percentages of LAB colony types during Nem Chua fermentation**

The form and the variation in percentage of each colony type during fermentation were noted. In total, there was 5 types of lactic colonies appearing on the MRS agar. The preliminary confirmation of lactic bacteria was done on the results of microscopic and macroscopic analysis with Gram coloration, catalase reaction, gas producing test and mobility of bacteria. The biochemical and microbiological characteristics of existed colonies are presented in Table 3.3. Lactic colony dynamics is shown in Table 3.4.
Table 3.3  The biochemical and microbiological characteristics of LAB colonies in Nem Chua

<table>
<thead>
<tr>
<th>Colony types</th>
<th>Colony appearance</th>
<th>Morphological bacteria</th>
<th>Gram coloration</th>
<th>Catalase Test</th>
<th>Gas producing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Domed, color varies from opalescent to yellowish, matt surface, circular edge, size of less than 3mm</td>
<td>Cocci form tetrads or rods in chain</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Conic, ivory-white, circular edge, smooth surface, size of larger than 3mm</td>
<td>Yeast</td>
<td>+</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>III</td>
<td>Similar to small transparent granule with diameter of less than 0.5mm</td>
<td>Cocci</td>
<td>+/-</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>IV</td>
<td>Flat, circular edge, round as a button (about 2mm) opalescent</td>
<td>Cocci</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>Domed but more prominent at central point, wrinkled edge that was more transparent than that in the center, smooth surface</td>
<td>Rods or coccoids</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

(N = not identified ; +/- = various)

The analysis of variance for percentages of each LAB colony types calculated during Nem Chua’s fabrication showed the significant difference between time examined (p < 0.05). Generally, from five colony types at the beginning, there was only 1 or 2 types at the end of fermentation for all studied processing units. This significant variation should be due to the effect of pH reduction and the production of inhibitory substances produced by some LAB towards the other bacteria. The observation that LAB have some preserving effects dates back to the turn of the nineteenth century. According to the early research the organic acids from sugar fermentation were responsible for the good keeping quality of fermented foods. Few bacteria are able to grow at pH values achieved by the action of lactic acid bacteria (Mäyrä-Mäkinen and Bigret, 2005).

There was a significant difference between LAB colony types’ percentages from four processing units (p < 0.05). That should be due to their various receipts and fermentation types applied in Nem chua manufacture. According to Bourgeois and Larpent (1989), the form and the dimension of colony depend on different factors (e.g. culture medium, temperature and time of incubation, type of fermentation,…).

Identification of lactic acid bacteria

The results of the identification for 131 strains isolated from Nem chua are noted in Table 3.5. Among 131 isolates identified from Nem Chua, *Lb. brevis* and *Lb. plantarum* presented throughout Nem chua fermentation with the important percentages (i.e. 25.19% and 21.37% respectively). Then, there were *Leuconostoc mesenteroides dextranicum* (14.50%), *Pediococcus pentosaceus* (12.21%) and *Lactococcus lactis* (11.45%). The other LABs such
as *Lb. paracasei ssp paracasei, Lb. fermentum, Lb. cellobiosus, Lb. collinoides, Leuconostoc lactis* existed in low percentage (from 1.53% to 3.82%).

### Table 3.4 Dynamics in the percentages of lactic colony types during Nem Chua fermentation (% of the population)

<table>
<thead>
<tr>
<th>Fermentation type</th>
<th>Processing units</th>
<th>Colony types</th>
<th>N0</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ut Thang (n = 3)</td>
<td>I</td>
<td>10.76 ± 4.47</td>
<td>10.51 ± 2.01</td>
<td>25.14 ± 18.53</td>
<td>5.14 ± 0.76</td>
<td>4.76 ± 0.46</td>
<td>3.23 ± 1.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>25.51 ± 26.69</td>
<td>10.55 ± 10.92</td>
<td>4.03 ± 1.55</td>
<td>13.88 ± 7.52</td>
<td>15.63 ± 11.33</td>
<td>6.75 ± 7.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>5.29 ± 2.26</td>
<td>1.08 ± 1.81</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.64 ± 9.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>56.54 ± 17.56</td>
<td>77.59 ± 19.51</td>
<td>70.67 ± 20.01</td>
<td>80.86 ± 8.16</td>
<td>78.88 ± 8.52</td>
<td>83.68 ± 29.50</td>
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<tr>
<td>fermentation</td>
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<td>III</td>
<td>9.30 ± 3.60</td>
<td>1.74 ± 0.80</td>
<td>4.44 ± 1.24</td>
<td>0.98 ± 1.39</td>
<td>0.11 ± 0.24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>66.35 ± 9.97</td>
<td>88.91 ± 3.16</td>
<td>69.27 ± 2.68</td>
<td>91.70 ± 1.31</td>
<td>95.17 ± 1.83</td>
<td>82.07 ± 4.71</td>
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<tr>
<td>Giao Tho (n = 3)</td>
<td></td>
<td>I</td>
<td>54.48 ± 2.29</td>
<td>21.14 ± 6.14</td>
<td>2.22 ± 2.72</td>
<td>1.27 ± 1.01</td>
<td>9.69 ± 7.44</td>
<td>22.15 ± 3.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>8.92 ± 0.50</td>
<td>3.98 ± 4.73</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6.27 ± 3.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>0.23 ± 0.45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.18 ± 1.49</td>
<td>1.28 ± 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>34.03 ± 0.53</td>
<td>72.25 ± 7.44</td>
<td>96.46 ± 1.95</td>
<td>96.9 ± 3.95</td>
<td>82.32 ± 3.65</td>
<td>67.55 ± 2.61</td>
</tr>
<tr>
<td>Thu Duc (n = 3)</td>
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<td>57.19 ± 0.23</td>
<td>29.20 ± 12.92</td>
<td>4.83 ± 2.45</td>
<td>28.87 ± 4.46</td>
<td>31.68 ± 2.76</td>
<td>41.54 ± 6.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>11.74 ± 0.03</td>
<td>0.18 ± 0.52</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>5.01 ± 0.96</td>
<td>0.55 ± 1.22</td>
<td>1.00 ± 1.59</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>26.06 ± 3.70</td>
<td>69.75 ± 12.47</td>
<td>93.74 ± 4.72</td>
<td>69.98 ± 13.44</td>
<td>68.32 ± 4.67</td>
<td>58.28 ± 6.63</td>
</tr>
<tr>
<td>Spontaneous</td>
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<td>I</td>
<td>57.19 ± 0.23</td>
<td>29.20 ± 12.92</td>
<td>4.83 ± 2.45</td>
<td>28.87 ± 4.46</td>
<td>31.68 ± 2.76</td>
<td>41.54 ± 6.97</td>
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<tr>
<td>fermentation</td>
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<td>III</td>
<td>11.74 ± 0.03</td>
<td>0.18 ± 0.52</td>
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<td>0</td>
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<tr>
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<td>IV</td>
<td>5.01 ± 0.96</td>
<td>0.55 ± 1.22</td>
<td>1.00 ± 1.59</td>
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<td></td>
<td></td>
<td>V</td>
<td>26.06 ± 3.70</td>
<td>69.75 ± 12.47</td>
<td>93.74 ± 4.72</td>
<td>69.98 ± 13.44</td>
<td>68.32 ± 4.67</td>
<td>58.28 ± 6.63</td>
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</table>
### Table 3.5 – Lactic acid bacteria identified during Nem chua fermentation.

<table>
<thead>
<tr>
<th>Name of LAB</th>
<th>N0 (%)</th>
<th>N1 (%)</th>
<th>N2 (%)</th>
<th>N3 (%)</th>
<th>N4 (%)</th>
<th>N5 (%)</th>
<th>Total</th>
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<tbody>
<tr>
<td><strong>Lb. brevis</strong></td>
<td>4.00</td>
<td>3.05</td>
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<td>1.53</td>
<td>0.76</td>
<td>0.76</td>
<td>10.69</td>
</tr>
<tr>
<td><strong>Lb. plantarum</strong></td>
<td>0.76</td>
<td>0.76</td>
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<td>0.76</td>
<td>0.76</td>
<td>3.82</td>
</tr>
<tr>
<td><strong>Lb. collinoides</strong></td>
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<td>0.76</td>
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<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>Lb. fermentum</strong></td>
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<td>1.53</td>
<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
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<tr>
<td><strong>Lb. cellobiosus</strong></td>
<td>1.53</td>
<td>1.53</td>
<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>Le. mesenterodes dextranicum</strong></td>
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<td>0.76</td>
<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>Lb. paracasei</strong></td>
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<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
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<td><strong>Pediococcus pentosaceus</strong></td>
<td>1.53</td>
<td>1.53</td>
<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>Lactococcus lactis</strong></td>
<td>1.53</td>
<td>1.53</td>
<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>Ln. lactis</strong></td>
<td>0.76</td>
<td>0.76</td>
<td>1.53</td>
<td>2.29</td>
<td>0.76</td>
<td>0.76</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10.69</td>
<td>10.69</td>
<td>10.69</td>
<td>10.69</td>
<td>10.69</td>
<td>10.69</td>
<td>100.00</td>
</tr>
</tbody>
</table>

(N = number of strains identified)

A study carried out on the microbiological changes of an Italian dry fermented sausage – manufactured without starter cultures, showed the predominance of *Lb. sakei* during the ripening of this product (approx. 51.1% of 90 strains identified) (Conter et al, 2005). Furthermore, the identification of LAB isolated from the Greek fermented sausage showed that the majority of them were assigned to the species of *Lb. plantarum* (Drosinos et al, 2005). However, *Lactobacillus cellobiosus* and *Lactobacillus brevis* often presented in the fermentation of vegetable products as well as in that of dairy products (Roissart and Luquet, 1994).

**CONCLUSION**

The identification of initial lactic bacteria in Nem chua is an important step which could help to optimize the traditional manufacture of this fermented meat product. Further research is needed to characterize the properties which have an influence on the sensorial characteristics of the product, such as the determination of lactic acid production, proteolytic and lipolytic activity, and the production of inhibitory substances. Phenotypic characterization based on sugar fermentation pattern may not always provide sufficient basis for the reliable identification of LAB although it is a useful tool for presumptive classification. The identification with DNA probes is faster, easier and more reliable than classical methods. Restriction patterns of rRNA genes were also shown to be useful for species or even strain identification (Schleifer et al, 1992; Hayford et al, 1999; Sesenä et al, 2005).
REFERENCES
IMPROVED EXTRACTION OF OIL AND VITAMINS FROM OIL PALM FRUITS WITH SUPERCRITICAL CO₂

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ABSTRACT

In the residual from palm oil production by traditional screw pressing method remains a significant quantity of tocochromanols and carotenoids. The objective of the study was to investigate an alternative environmentally friendly extraction method, which could better recover these valuable minor compounds. The work included a study of extraction of palm mesocarp by supercritical carbon dioxide (SCCO₂) at different pressure, temperature and flow rate. Total oil yield was investigated in the course of extraction. Tocochromanols and carotene were evaluated not only in the extraction oil, but also in the residue fiber. Modeling of extraction process is also performed for further up-scaling. The results showed that with the flow rate of 35 (kg/h)/kg, oil could be recovered up to 80-90% after 120 minutes. Using SCCO₂ could recover much more tocochromanols and carotene than using traditional screw pressing method.

Keywords: Supercritical extraction, Palm oil, Minor content, Mathematical modeling

INTRODUCTION

Palm oil is well known all over the world because of its high quality. This oil is commonly produced by using both wet and dry system to extract the crude oil from the digested fruit mash [1]. However, a significant quantity of carotenoids and tocochromanols, which can be used as vitamin E and pro-vitamin A, remain in the residual oil extracted from palm press fibers [2]. The objective of the study is therefore to investigate an extraction method, which can recover beneficially these valuable minor compounds. Supercritical fluid extraction (SFE) is an environmentally friendly method because it doesn’t release dangerous solvent into the atmosphere. This technique has been successfully investigated for many types of agro-materials. In this work supercritical carbon dioxide (SCCO₂) was selected to extract palm mesocarp at different pressure, temperature and flow rate. In order to compare the efficiency with traditional screw pressing method, tocochromanols and carotene are investigated not only in the extraction oil, but also in the residue fiber.

MATERIALS AND METHODS

Sample preparation

Palm fruits (Elaeis guineensis) are separated into skin, mesocarp and kernel. The yellow part of the mesocarp is investigated. The particle size of the pulp ready for supercritical extraction is about 1 x 2 x 6 (mm x mm x mm).

Equipment and experiment procedure

The extraction unit is described as Figure 1. The 100ml steel extractor cell is loaded with 14.5 g of palm mesocarp (fixed bed) for each experimental run. The gas flow rates are 0.2 and 0.5 kg/h (equivalent to 14 and 35 (kg/h) of gas per kg of sample). The extracts are collected continuously in 10ml glass vials used as sample collector.
Analytical method

High Performance Liquid Chromatography (HPLC) system from Gynkotek company with RF 1002 Fluorescent detector was used for the analysis. Tocopherols and tocotrienols (tocochromanols) in the oil samples were separated on a LiChrosorb Diol 5 μm, 250 x 4.6 mm column (Chrompack No 612834). The mobile phase is Hexane (96%) and Butyl-methyl-ether (4%) at the flow rate of 1300 μL/min. Injection volume is 20 μL.

UV-Vis Spectroscopy (spectrometer UV-120-02 from Shimadzu) was used to determine the content of carotene in analyzed sample by measuring the absorbance (ABS) at 450 nm. An amount of 10 to 20 mg of oil sample is diluted with 2 ml mixture of acetone and hexane (30:70 by Vol. %).

Soxhlet extraction method was used to extract the total oil of original palm mesocarp and the residue from the supercritical CO2 extraction.

Mathematical modeling

Sovova (1994) [3] model was tested to describe the kinetic behavior of SCCO2 extraction. The experimental data were fitted using procedure reported in Martinez et al. (2003) [4]

RESULTS AND DISCUSSION

Effect of Pressure

As a result of changing fluid density and solvate power, the solubility of oil in dense fluid is varied with pressure [5]. The total palm oil yields therefore are significantly affected by pressure. Following the condition described in Figure 2, increasing pressure from 200 to 400 bar could increase oil yield from 35 to 47% after 120 minutes of extraction, while at first 60 minutes the yield’s difference is much larger. In general, at any temperature extraction yield will increase with higher pressure.
**Effect of temperature**

At high pressure (300 or 400 bar), extraction yield increases with higher temperature (Figure 3). This phenomenon is explained by the fact that the effect of palm oil vapor is more pronounced than the effect of decreasing solubility when changing extraction temperature [5]. At lower pressure (200 bar), for the first 60 minutes of extraction time, lower temperature is more favorable for the extraction. However, when the available oil near the surface is depleted, the mass transfer of oil from the center of the pulp fruit to the surface is the main factor to determine the extraction rate of the supercritical extraction. In consequence, higher mass transfer at 55°C gives a better extraction yield after 60 minutes of extraction.

![Fig. 2. Extraction of mesocarp at 45°C, 14 (kg/h)/kg](image1)

![Fig. 3. Extraction of mesocarp with SCCO₂ at 14 (kg/h)/kg](image2)
Effect of flow rate
The flow rate affects the oil loading ability of the solvent and mass transfer in the palm pulp. Figure 4 shows obviously that an increase in flow rate will increase amount of the collected oil, therefore shorten the extraction time. However, the balance between the addition of oil recovery and the cost of fluid and power consumption should be considered in the economical point of view.

![Figure 4. Extraction of mesocarp with SCCO$_2$ at 55$^\circ$C](image)

Mathematical modeling
Data above give a note that extraction at 200 bar is not sufficient for oil recovery, even at very high flow rate. Consequently, SCCO$_2$ at 400 or 300 bar with flow rate of 35 (kg/h)/kg will be further investigated at different extraction temperatures. The pseudo steady state model of Sovova (1994) [3] is applied to describe the modeling of the extraction process (Figure 5).

![Figure 5. Comparison of experimental data with fit curves at 35 (kg/h)/kg, (♦) 300 bar, 45$^\circ$C; (▲) 300 bar, 65$^\circ$C; (■) 400 bar, 45$^\circ$C; (---) Modeling curves](image)
It can be observed that the experimental data are fitted well with this Sovova model. Palm oil extraction with SCCO$_2$ can be divided clearly with 3 stages with different functions. In the first stage (Constant Extraction Rate, CER), where the oil is easily accessible throughout the fixed bed, the extraction curve is a straight line through the origin. When the easily accessible oil becomes exhausted at the fluid entrance, a transition period between the fast and slow extraction periods begins. In this period, called the Falling Extraction Rate period (FER), the easily accessible oil is still extracted in one section of the fixed bed, while the extraction from the inside of the particles takes place in the other section. The boundary between the two sections described above, passes through the bed until it reaches its end. After the boundary has reached the end of the fixed bed the Diffusion Controlled Extraction Rate period (DCER) comes with extraction rate controlled by diffusion of the oil from inside of the particles to outside. Parameters of Sovova model described as Figure 5 are presented in Table 1. The first extraction period is characterized by $t_{CER}$ (time of extraction till the end of constant extraction rate period). The other important parameters are $k_{YA}$ and $k_{XA}$, which are mass transfer coefficient in fluid phase and solid phase respectively.

Table 1. Parameters adjusted for SFE extracts from palm mesocarp with Sovova model

<table>
<thead>
<tr>
<th>Pressure (bar)</th>
<th>Temperature (°C)</th>
<th>t$_{CER}$ (min)</th>
<th>$Q_{CO2}$ $10^5$ (kg/s)</th>
<th>$k_{YA}$ $10^3$ (s$^{-1}$)</th>
<th>$k_{XA}$ $10^4$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>45</td>
<td>24</td>
<td>13.8</td>
<td>9.4</td>
<td>14.5</td>
</tr>
<tr>
<td>300</td>
<td>65</td>
<td>17</td>
<td>13.8</td>
<td>12.8</td>
<td>15.3</td>
</tr>
<tr>
<td>400</td>
<td>65</td>
<td>15</td>
<td>13.8</td>
<td>10.8</td>
<td>13.2</td>
</tr>
</tbody>
</table>

The result shows that with conditions applied, total yield could be up to 35 – 42% (equivalent to 80 – 90% of oil recovery) after 120 minutes of extraction run. The values of $k_{YA}$ are always higher than those of $k_{XA}$. The different between these mass transfer coefficients shows that the effect of convection in the fluid phase is more important than the effect of diffusion in the solid phase. However, the mass transfer coefficients $k_{XA}$ in this palm oil extraction process is rather higher than those in other natural compound extraction reported elsewhere [3], [4]. This agrees with the visible observation that palm oil can easily diffuse from inside to the outer- face of the palm fiber.

Tocochromanols and carotene extraction

Tocochromanols and carotene are interesting valuable minor components in supercritical fluid extraction [6], [7]. Figure 6 shows that using SCCO$_2$ could co-extract these compounds with the concentration in the same range of normal commercial processing palm oil.

Commonly the composition of palm mesocarp varies with the size and the self-life of palm fruits. Thus, to objectively evaluate the efficiency of recovery of tocochromanols and carotene by different extraction techniques, the relative comparison of the concentration of these compounds in the extracted oil and in the residue oil is used (Table 2). Enrichment factor $K$ of component X is defined as following equation:

$$K(X) = \frac{\text{concentration of component X in the extracted oil}}{\text{concentration of component X in the residue oil}}$$
As a result, the extraction technique with higher value of $K(X)$ gives a better potential to recovery component $X$.

![Graph showing Carotene and Tocochromanols Concentration]

**Fig. 6.** Minor content in extracted and residue oil with SCCO$_2$ at 400 bar, 55°C, 35 (kg/h)/h

**Table 2.** Enrichment factor of Tocochromanols and Carotene with different SFE

<table>
<thead>
<tr>
<th>Technique</th>
<th>$K$(carotene)</th>
<th>$K$(tocochromanols)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCO$_2$, 300 bar, (45°C - 65°C)</td>
<td>0.79 - 1.21</td>
<td>0.41 - 0.67</td>
</tr>
<tr>
<td>SCCO$_2$, 400 bar, (45°C - 65°C)</td>
<td>0.90 - 1.19</td>
<td>0.54 - 0.86</td>
</tr>
<tr>
<td>Screw pressing calculate after (Choo, 1996)</td>
<td>ca. 0.12</td>
<td>ca. 0.27</td>
</tr>
</tbody>
</table>

The result in Table 2 shows obviously that, using SCCO$_2$ could recover much more tocochromanols and carotene than using traditional screw pressing method.

**CONCLUSION**

When the target is recovery of oil with valuable minor compounds like tocochromanols and carotene, SCCO$_2$ extraction of palm fruit could become an alternative method for the traditional screw pressing.

**REFERENCES**


IMPROVING THE PROCESS OF PRODUCING CHITOSAN FROM SHRIMP SHELL

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\textsuperscript{b}Ho Chi Minh City University of Technology, Vietnam

ABSTRACT

By the survey and experimentation, we carried out isolation chitosan from shrimp shells. To remove the minerals from shrimp shell, we soaked dried shrimp shell into solution of hypochloride acid (HCl) 6\% for 6 hours. The process of deacetylation carried out by sodium hydroxide (NaOH) 7M at the temperature of 130\degree C-160\degree C. The chitosan product has white colour or ivory-coulored and dissolves completely in solution of acetic acid 1.5\%

INTRODUCTION

In the nature, chitin and chitosan don’t exist severally, they often combines to other substances. To isolate chitosan, the people usually use the chemical methods. In general, crustacean shells cooked in the solution of sodium hydroxide for 1 hour, and removed the minerals by soaking the shell into the diluted acid solution for 24 hours. The collected chitin is deacetylated by the concentrated solution of sodium hydroxide (40-45\%) at the boiled temperature.

The scientists conducted many methods of isolation of chitin, chitosan from shrimp shells such as:

* The method of Hackman, 1954 (producing chitin from lobster shrimp shell): Shell - washing - drying (100\degree C) - soaking (HCl 12N, room temperature, 5hrs) - washing - drying (100\degree C) - grinding - shaking (HCl 2N, 48hrs) – centrifuge - cooking (NaOH, 100\degree C, 12hrs) - washing - centrifuge - drying - chitin.


These methods have weak-points such as a long time of production, the process not simple, spending a lot chemical. The Japanese scientists recently proposed the method of producing chitosan without the chitin isolating stage. There are: Shell - washing - drying – removing minerals (HCl 2M, 1hr, 120\degree C) – washing – removing protein and deacetylation (NaOH, 15M, 150\degree C, 1hrs) – washing - drying - chitosan.

In Vietnam, the method of Japanese applied and changed by Nguyen Huu Duc and his collaborators, 1994. There are: Shell - washing - drying – removing minerals (HCl 3.5M, 6hrs,

The objective of this work was to study for improving the process of producing chitosan from shrimp shells base on the above methods.

MATERIALS AND METHODOLOGY

Materials

The material for isolating chitosan is the black tiger shrimp shell, which have a lot in the fisheries frozen factories.

All chemicals were purchased in analytical grade from chemical companies and used as received.

Methodology

The experimental procedure of isolating chitosan has following stages:

* Treatment of shrimp shells: The black tiger shrimp shell wastes received from the factory are washed to remove extraneous matters and organic substances. Taking the shell and drying it and calculating the norm of drying.

* Removing the minerals: the dried shrimp shell is soaked into solution of hypochloride acid (HCl) at the concentrations of 2% ÷ 12% for 3 ÷ 24 hrs (at room temperature). After soaking, the samples are washed and dried. For testing minerals remained in the samples, we immersed the sample into HCl 15%. If there are gas foams appearing on the surface of sample, the process of removing minerals from the shell is not completely.

* Deacetylation: the samples after removing minerals deacetylated by solution of NaOH 5 ÷ 11M for 30 ÷ 120 minutes at the temperature of 130 ÷ 160°C. To test deacetylation, we took the treated samples to wash off NaOH and immerse it into solution of acetic acid 1.5%. If the sample is dissolved completely, the sample has a degree of deacetylation above 50%.

After choosing the technological parameters of producing chitosan from shrimp shell, we carried out surveying some parameters of chitosan product.

Influence of concentration of acetic acid solution on dissolving chitosan: we dissolved chitosan into acetic acid of different concentrations (0.5 ÷ 2.5%) to define the dissolvable time and level of chitosan.

To establish the relation between concentration of chitosan and the viscosity of chitosan solution: we measured the viscosity of chitosan solution of 0.25% ÷ 3.25% (concentration of acetic acid solution was chosen before).

Calculation of production norm

We weigh the weight of sample before and after of each stage of the production. The norm of each stage is calculated by the following formula:

\[ a = \frac{b}{c} \]

Where:

a: the norm of stage; b: the weight of sample before the stage (kg)

The weight of sample after the stage (kg)
Measurement of colour of chitosan
We used the colour system of Yxy of chromameter CR-200 (Minolta). The activity principles of CR-200 bases on absorbing reflection of light from the surface of sample. The result is shown on the screen of the equipment.

Degree of Deacetylation
Degree of deacetylation (DD) was determined followed method of base on total of nitrogen. When chitin transforms to chitosan, based on the change of % nitrogenous total, DD was determined followed of the equation:

$$DD = \frac{c-a}{b-a} \times 100\%$$

where:

c: total of nitrogen in chitosan sample (%)
a: total of nitrogen in chitin calculated followed theory (6.89%).
b: total of nitrogen in chitosan calculated followed theory (8.69%).

Moisture and ash content of chitosan
Moisture content was determined followed method of drying at the temperature of 105°C to the constant weight (TCVN 3700-90).
Ash content was determined followed method of make white-hot at the temperature of 550-600°C (TCVN 5105-90).

Measurement of the viscosity
The viscosity of chitosan solution was measured by Brookfield DV-I+Viscometer. Measurement was carried out at speed of the spindle of 6 rounds per minute for 3 minutes.

Statistical analysis
Data were initially evaluated using analysis of variance (ANOVA, Statgraphics version 7.0 program). When the ANOVA test indicated a significant (p<0.05) difference among means, a least significant difference test (LSD) was used to identify which film means differed significantly.

Analysis of regression
We use the method of analysis of coefficients of regressive equation to represent the relation between concentration of chitosan and the viscosity of chitosan solution.

RESULTS AND DISCUSSION
Removing the minerals
According to Hackman, 1954, the inorganic components are usually removed by inorganic acid solutions. Calcium carbonate is a main component of shrimp shell.
Removing mineral from the shrimp shell is a process of reaction between hypochlorides acid and calcium carbonate:

$$CaCO_3 + 2HCl = CaCl_2 + H_2O + CO_2$$
In addition, hydrochloride acid also reacts with other mineral of shrimp shell:

\[ \text{P}_2(\text{CO}_3)_5 + 10\text{HCl} = 2\text{PCl}_5 + 5\text{H}_2\text{O} + 5\text{CO}_2 \]

During the reaction occurring, there is a gas of carbonic flying off. This is the signal to recognize the minerals remaining or not in the shell.

The result of removing minerals is shown in the chart 1:

![Chart 1: effect of concentration (%) of hypochloride acid solution and the time (hrs) on ability of removing mineral.](chart)

From the chart 1, the time to remove minerals from shrimp shell is 10 hrs for the treatments HCl of 2% and 3%, 8 hrs for 4% and 5%, and 6 hrs for 6% and 5 hrs for 7% ÷ 12%. It is necessary to have enough time for absorb of acid into the shell. To choose concentration and time for removing mineral, it depends on situation of producing chitosan. If we have time, we can use low concentration (2%) of acid. If we have no time, we can use acid of 5% or 6%.

**Deacetylation**

According to the studies of some scientists, protein is removed from shell by hot water at low level and by hydroxide sodium at higher level. When pH of solution increasing up to 9 effects to remove protein from the shell (Hackman, 1954). In addition, chitin will be deacetylated to form chitosan when it is treated by hydroxide sodium solution at high temperature and concentration. Meyers and Weherli, 1960 received the product, which was eliminated off almost acetyl groups by NaOH 50% at 100°C for 35-40 minutes.

In the limit of study, we couldn’t research degree of deacetylation on angle of number of acetyl groups eliminated that is on dissolvable of chitosan.
The results of experiment are shown in table 1:

Table 1: effect of hydroxide sodium concentration \((C_M)\) and time of treatment on dissolvable of chitosan

<table>
<thead>
<tr>
<th>(C_M)</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>8</td>
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<tr>
<td>11</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Notes:
(-) undissolvable in acetic acid 1.5%
(+ ) little dissolvable in acetic acid 1.5%
(++) not completely dissolvable in acetic acid 1.5%
(+++) completely dissolvable in acetic acid 1.5%

From the table 1, the samples of 5M and 6M aren’t completely dissolvable in acetic acid 1.5% for all the times. For the times of 30 and 45 minutes, the samples aren’t completely dissolvable at all concentrations of hydroxide sodium. For the times of 60 and 75 minutes, the samples dissolved well at concentration above 9M. This difference isn’t clear. However, the samples of above 7M and 90 minutes are completely dissolvable in acetic acid 1.5%.

According to some documents, chitosan is a polysaccharide consisting of molecules of D-glucosamine and maximum of 30% acetyl group. For deacetylization, it is necessary to make weak of the link between amino and acetyl groups of chitin. Therefore, it needs a certainly energy for deacetylization. It means that the time has enough.

Besides the dissolvable, we also compared the colour of chitosan product. The results are shown in the table 2:

Table 2: effect of hydroxide sodium concentration \((C_M)\) and time of treatment on colour of chitosan

<table>
<thead>
<tr>
<th>(C_M)</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>72.04</td>
<td>65.93</td>
<td>66.96</td>
<td>68.58</td>
<td>59.03</td>
<td>51.73</td>
<td>60.07</td>
</tr>
<tr>
<td>6</td>
<td>65.20</td>
<td>56.03</td>
<td>60.91</td>
<td>55.07</td>
<td>48.12</td>
<td>47.57</td>
<td>52.26</td>
</tr>
<tr>
<td>7</td>
<td>37.91</td>
<td>41.43</td>
<td>41.74</td>
<td>45.36</td>
<td>57.79</td>
<td>47.19</td>
<td>58.34</td>
</tr>
<tr>
<td>8</td>
<td>47.33</td>
<td>52.01</td>
<td>53.91</td>
<td>44.72</td>
<td>49.94</td>
<td>53.65</td>
<td>51.80</td>
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<tr>
<td>9</td>
<td>49.90</td>
<td>53.32</td>
<td>47.62</td>
<td>60.73</td>
<td>46.81</td>
<td>46.51</td>
<td>52.17</td>
</tr>
<tr>
<td>10</td>
<td>44.09</td>
<td>41.15</td>
<td>43.88</td>
<td>39.37</td>
<td>48.01</td>
<td>50.09</td>
<td>51.66</td>
</tr>
<tr>
<td>11</td>
<td>38.58</td>
<td>49.64</td>
<td>42.07</td>
<td>41.53</td>
<td>49.03</td>
<td>46.12</td>
<td>46.22</td>
</tr>
</tbody>
</table>
The result of statistical analysis showed that time of treatment and concentration of hydroxide sodium effecting on colour of chitosan (p<0.05). To combine the result of table 1 and 2, we chose regulation of treatment of NaOH 7M for 90 minutes.

**Effect of acetic acid solution concentration on dissolvable of chitosan product**

To dissolve 2% chitosan into acetic acid solution of 0.5% -2.5%. The dissolvable times of chitosan are shown in the chart 2:

![Chart 2: Effect of acetic acid solution concentration on dissolvable of chitosan](image)

The result of statistical analysis showed that the acetic acid concentration effecting on dissolvable of chitosan (p<0.05). By LSD test, we have the difference between the treatments, there are: 0.5d – 1c – 1.5b – 2b – 2.5a. Means with different superscripts (a-d) are significantly different (p<0.05).

**The relation between concentration of chitosan and the viscosity of chitosan solution**

To dissolve chitosan with different concentrations into acetic acid solution of 1.5%. The relation between concentration of chitosan and the viscosity of chitosan solution is represent by the following equation: \( y = 95.703x^2 - 125.49x + 59.407 \) with \( R^2 = 0.9996 \)

![Chart 3: The relation between concentration of chitosan and the viscosity of chitosan solution](image)
The process of producing chitosan from shrimp shell

- Shrimp shell
- Washing – drying
- HCl 6%, 6hrs, room temperature (w:v = 1:8)
- Washing
- NaOH, 7M, 130°C-160°C, 90minutes (w:v = 1:8)
- Washing – drying
- Chitosan product

Chart 4: Diagram of process of producing chitosan from shrimp shells

The chitosan product has white color, in plate form, the degree of deacetylation of 90.73%, moisture content of 16.69% and ash content of 0.153%.

The production norm is about 44

Here are some pictures of producing chitosan from shrimp shell:

picture 1: dried shrimp shell

picture 2: removing minerals

picture 3: deacetylation
CONCLUSION
With the suggestible process, we hope to save on the time and chemical substances used in production of chitosan from shrimp shells.

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SOME FACTORS INFLUENCING ON THE DISRUPTIVE PRESSURE OF CHITOSAN FILM

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ABSTRACT

Some factors affecting on the disruptive pressure of chitosan film were investigated. The disruptive pressure of film was determined to be $3.23 \text{ kG/cm}^2 \div 2 \text{ kG/cm}^2$ at concentration of acetic acid solution of $1\% \div 3.5\%$, and to be $1.97 \text{ kG/cm}^2 \div 4.21 \text{ kG/cm}^2$ at chitosan ratio of $2\% \div 4\%$, and to be $1.72 \text{ kG/cm}^2 \div 3.45 \text{ kG/cm}^2$ depending on film’s composition.

INTRODUCTION

Chitosan, which isolated from shrimp shell has been studied to apply in the different fields of industry, agriculture, medicine, food, etc. Chitosan film has strong points in applying in food industry and medicine. However, the physical properties of chitosan film haven’t been studied a lot yet. The scientists interest studying the mechanic durability of chitosan film in packaging and transportation food.

The scientists are interesting chitosan film in its make and application. Chitosan film can be used to reduce the effects of the surroundings into food.

The objective of this work was to study some factors influencing on the disruptive pressure of chitosan film. There are the parameters of drying film, the solvent and concentration, the ratio of chitosan, the additives, the environmental temperature and shelf life of chitosan film.

MATERIALS AND METHODOLOGY

Materials

Chitosan isolated from black tiger shrimp shell waste which have a lot in the fisheries frozen factories, obtained from the technology lab will be used throughout the study. The specific properties of chitosan sample are characterized within the following parameters: form, color, moisture content, ash content, solubility, and degree of deacetylation. The chitosan has white color, in powder form, the degree of deacetylation of $90.73\%$, moisture content of $16.69\%$ and ash content of $0.153\%$.

All chemicals were purchased in analytical grade from chemical companies and used as received.

Methodology

Characterization of chitosan

Moisture and ash content

Moisture content was determined followed method of drying at the temperature of $105^\circ\text{C}$ to the constant weight (TCVN 3700-90).
Ash content was determined followed method of make white-hot at the temperature of 550-600°C (TCVN 5105-90).

**Degree of Deacetylation**

Degree of deacetylation (DD) was determined followed method of base on total of nitrogen. When chitin transforms to chitosan, based on the change of % nitrogenous total, DD was determined followed of the equation:

$$ DD = \frac{c-a}{b-a} \times 100\% $$

where:
- c: total of nitrogen in chitosan sample (%)
- a: total of nitrogen in chitin calculated followed theory (6.89%).
- b: total of nitrogen in chitosan calculated followed theory (8.69%).

**Film formation**

The chitosan films were prepared by dissolving chitosan into the solvent and spreading the chitosan solution (50ml) on the glass plate (210x297mm) and dried. The solvents, the additives, content of solvent, additive and chitosan were changed following the experiments. Chitosan films formed were used to test the disruptive pressure in the experiments:

I. Preliminary experiments were conducted using dry temperature of 40, 50, 60, 70, 80 and 90°C to find out dry times correlative (the other experimental factors were stable). Films prepared from dry temperature and time of 50°C/2h00, 60°C/1h45, 70°C/1h30, and 80°C/1h15 were determined as the feasible ones. Then experiment was carried out to choose the parameters for drying film.

II. Preliminary experiments were conducted using solvents of formic acid, acetic acid and citric acid. Film prepared from acetic acid was determined as the highest disruptive pressure. Then experiments were conducted using acetic acid concentration of 1, 1.5, 2, 2.5, 3, and 3.5%. (drying in system I)

III. Experiments were conducted using chitosan ratios of 2, 2.5, 3, 3.5 and 4% to dissolve in acetic acid of II

IV. Experiments were conducted using chitosan films from III and keeping at temperatures of -25 °C, -30°C, -15 °C, -20°C, 5 °C, 25 °C, 30 °C for 10, 20, 30 and 40 days.

V. Experiments were conducted using additives of regenerate starch, guaran gum, regenerate starch + PEG400, guaran gum + PEG400 and none additive.(each additive of 1% and PEG400 of 0.4%)

**Film thickness**

Thickness of films was measured with a digital micrometer (Mega-cheek 5F) at 7 random positions on film. The disruptive pressure property of chitosan film was calculated based on average thickness.
**Disruptive pressure**

The disruptive pressure of chitosan film was determined basing on the stand of film under the pressure. The test film was sealed to a glass jar with the diameter of 4cm and the jar was placed in the penetrometer. The penetrometer has a cylinder with diameter (D) of 0,8cm to test. The test process is carried by pressing the cylinder on the film until the film will be broken. The value of force (F) to break film is showed on the Penemeter’s screen. The break pressure (P) of chitosan film is calculated from the following equation:

\[ P = \frac{F}{S} \]

Where:
- P is the disruptive pressure (kG/cm²)
- F is the break force (kG).
- S is area of the cylinder (cm²)

\[ S = \left( \frac{D}{2} \right)^2 \pi \]

- D is diameter of cylinder (cm)
- \( \pi \) is 3.14

**Statistical analysis**

Data were initially evaluated using analysis of variance (ANOVA, Statgraphics version 7.0 program). When the ANOVA test indicated a significant (p<0.05) difference among means, a least significant difference test (LSD) was used to identify which film means differed significantly.

**RESULTS AND DISCUSSION**

The disruptive pressure concerns to the firm of film. The more film is strong the more disruptive pressure is high. It depends on structure of polymer, the condition of formation of film, etc. (Stephane Guilbert Nathalie Gontard and Leon G.M.Gorris, 1996)

**Effect of dry temperature and time on the disruptive pressure of chitosan films**

The dry temperature and time effect structure and physical properties of film. The chitosan films were prepared by dissolving 3% chitosan into acetic acid of 1.5% and spreading the chitosan solution (50ml) on the glass plate (210x297mm) for drying. The results of the experiment are shown in table 1.

Dry temperature and time didn’t effect to thickness of film. By drying, water and solvent are removed. This process effects to the arrangement of molecules in the polymer chain, but it didn’t change considerably thickness of film.

The disruptive pressure of chitosan film is affected by dry temperature and time. The disruptive pressure of the film from treatment of 80°C/1h15 is lowest (1,14 kG/cm²) and differs significantly from remain treatments. It could be caused of high temperature influencing to structure of chitosan film.
Table 1: Effect of dry temperature and time on the disruptive pressure of chitosan film

<table>
<thead>
<tr>
<th>Drying</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C/2h00</td>
<td>25.34 ± 0.58a</td>
<td>2,11 ± 0.14b</td>
</tr>
<tr>
<td>60°C/1h45</td>
<td>24.69 ± 0.45a</td>
<td>2.24 ± 0.28b</td>
</tr>
<tr>
<td>70°C/1h30</td>
<td>25.58 ± 0.20a</td>
<td>2.37 ± 0.22b</td>
</tr>
<tr>
<td>80°C/1h15</td>
<td>25.15 ± 0.33a</td>
<td>1.14 ± 0.02a</td>
</tr>
</tbody>
</table>

Means with different superscripts (a-b) are significantly different (p<0.05).

The films dried at 50°C/2h00, 60°C/1h45 and 70°C/1h30 have no significant difference on the disruptive pressure. Drying of 70°C/1h30 was chosen as the next experiments due to dry time be short and the disruptive pressure be highest (2,37 kG/cm²).

Effect of acetic acid concentration on the disruptive pressure of chitosan film

The chitosan films were prepared by dissolving 3% chitosan into acetic acid with the changed contents and spreading the chitosan solution (50ml) on the glass plate (210x297mm) and dried at 70°C/1h30. The results of the experiment are shown in table 2.

Table 2: Effect of acetic acid concentration on the disruptive pressure of chitosan film

<table>
<thead>
<tr>
<th>Content (%)</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.23 ± 0.48a</td>
<td>3.23 ± 0.25b</td>
</tr>
<tr>
<td>1.5</td>
<td>25.45 ± 0.94a</td>
<td>3.06 ± 0.26b</td>
</tr>
<tr>
<td>2</td>
<td>26.13 ± 1.08a</td>
<td>2.84 ± 0.25ab</td>
</tr>
<tr>
<td>2.5</td>
<td>24.84 ± 0.24a</td>
<td>2.52 ± 0.49ab</td>
</tr>
<tr>
<td>3</td>
<td>26.15 ± 0.35a</td>
<td>2.33 ± 0.17ab</td>
</tr>
<tr>
<td>3.5</td>
<td>25.98 ± 0.55a</td>
<td>2.00 ± 0.23a</td>
</tr>
</tbody>
</table>

By the result of ANOVA, thickness and disruptive pressure of chitosan films from acetic acid concentration treatments aren’t different (p>0.05). The water and solvent were removed during drying, there is only the chitosan identical remaining on the glass plate between the treatments. Testing of least significant difference (LSD) was showed the disruptive pressure of treatments of 1% and 1.5% acetic acid having difference with 3.5% acetic acid. However, the treatment of 1.5% acetic acid was chosen due to chitosan dissolved well at this acid concentration and not affected by acid smell after forming film.

Effect of ratio of chitosan on the disruptive pressure of chitosan film

The results of the experiment are shown in table 3.

Table 3: Effect of ratio of chitosan on the disruptive pressure of chitosan film

<table>
<thead>
<tr>
<th>Factors (%)</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.55 ± 0.37a</td>
<td>1.97 ± 0.23a</td>
</tr>
<tr>
<td>2.5</td>
<td>17.05 ± 0.15b</td>
<td>3.16 ± 0.35ab</td>
</tr>
<tr>
<td>3</td>
<td>25.18 ± 0.39c</td>
<td>3.45 ± 0.14b</td>
</tr>
<tr>
<td>3.5</td>
<td>30.96 ± 0.99d</td>
<td>4.15 ± 0.38b</td>
</tr>
<tr>
<td>4</td>
<td>38.89 ± 1.02c</td>
<td>4.21 ± 0.74b</td>
</tr>
</tbody>
</table>

Means with different superscripts (a-e) are significantly different (p<0.05).
The thickness of films is significantly different between treatments. This difference is clearly due to forming film by the same solution volumes on the same glass plate areas. The thickness is mainly determined by quantity of chitosan.

The disruptive pressure of film is also affected by concentration of chitosan. This difference is to have statistical signification. The more concentration is high, the more the thickness is high conducting the firm of film better.

Although the disruptive pressure of treatment of 3.5% and 4% (4.15kG/cm² and 4.21 kG/cm²) is higher the disruptive pressure of treatment of 3% (3.45kG/cm²) but this difference isn’t significant. The treatment of 3% chitosan was chosen.

**Effect of medium temperature and time on the disruptive pressure of chitosan film formed from different concentrations of chitosan**

The chitosan films were prepared by dissolving 2, 2.5, 3, 3.5, 4% chitosan into acetic acid 1.5% and spreading the chitosan solution (50ml) on the glass plate (210x297mm), dried at 70°C/1h30. The results of the experiment are shown in table 4.

Table 4: Effect of medium temperature, time and chitosan concentration on the disruptive pressure of chitosan film

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ÷ 30</td>
<td>24.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4029&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 ÷ 10</td>
<td>24.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5759&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>-15 ÷ -20</td>
<td>24.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6025&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>-25 ÷ -30</td>
<td>24.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6297&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3968&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>24.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4462&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>24.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5347&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>24.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6645&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>24.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7187&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Content of chitosan (%)</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3930&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5</td>
<td>18.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2818&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>24.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.7395&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.5</td>
<td>30.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.8812&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>37.80&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.4683&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different superscripts (a-e) are significantly different (p<0.05).

From the result of ANOVA, three factors of temperature, time and chitosan concentration have no interaction significantly (p>0.05) on thickness of film. Temperature and time don’t affect thickness of film but chitosan concentration effects.

There are interactions between three factors of temperature, time and chitosan concentration on the disruptive pressure of chitosan films.
The stableness of film is affected by temperature. The more temperature decreases, the more the disruptive pressure of film is high. It could be structure of film more firmly at temperature more lowly.

At the beginning time after forming film, the disruptive pressure of chitosan film is lowest (3.3968kG/cm²) and highest after 40 days (3.7187G/cm²). After forming film, it needs to have a necessary time for stableness of structure of film.

The disruptive pressure of chitosan films are different significantly between the treatments of concentration. The more concentration increases, the more the disruptive pressure of film is high.

**Effect of the additives on the disruptive pressure of chitosan film**

The chitosan films were prepared by dissolving 3% chitosan and the different additives (1%) into acetic acid 1.5% and spreading the chitosan solution (50ml) on the glass plate and dried at 70°C/1h30. The results of the experiment are shown in table 5:

<table>
<thead>
<tr>
<th>Additives</th>
<th>Thickness (μm)</th>
<th>The disruptive pressure (kG/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29.28 ± 0.88b</td>
<td>3.15 ± 0.19bc</td>
</tr>
<tr>
<td>B</td>
<td>28.67 ± 1.35ab</td>
<td>2.26 ± 0.37a</td>
</tr>
<tr>
<td>C</td>
<td>31.66 ± 1.26b</td>
<td>3.45 ± 0.14c</td>
</tr>
<tr>
<td>D</td>
<td>30.52 ± 1.48b</td>
<td>2.49 ± 0.03ab</td>
</tr>
<tr>
<td>E</td>
<td>25.02 ± 0.77a</td>
<td>1.72 ± 0.26a</td>
</tr>
</tbody>
</table>

Means with different superscripts (a-b) are significantly different (p<0.05).

Note:
A. Guaran gum
B. Regenerate starch
C. Guaran gum+PEG400 (polyethylene glycol)
D. Regenerate starch+PEG400
E. None additive

Thickness between none additive treatment (E) and others is different significantly.

The disruptive pressure of chitosan films aren’t different significantly between the treatments of A, D and B. It could be quantity of these additives not enough. The treatment of Guaran gum+PEG400 has the highest disruptive pressure (3.45 kG/cm²) and differs from others. Guaran gum and PEG400 effect to the thick and the firm of chitosan films.

**CONCLUSION**

The disruptive pressure and thickness of chitosan film are increased depending on chitosan concentration. The environmental condition of temperature and time didn’t effect on thickness but to effect on the disruptive pressure of chitosan film. The additives added into films increases thickness and the disruptive pressure of films.
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THERMAL MECHANICAL COMPRESSION TEST (TMCT): MEASUREMENT OF PHASE TRANSITION TEMPERATURE OF RICE FLOUR

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ABSTRACT

TMCT (Thermal Mechanical Compression Test) was applied to measure the Tg-r (glass-rubber transition temperature) of rice flour at low moisture content (2.4-19.5% wet basis). As expected, Tg-r decreased with increasing moisture content. The Tg-r temperatures measured by TMCT were comparable with those measured by DSC (Differential Scanning Calorimetry), TMA (Thermo-mechanical Analysis), and DMTA (Dynamic Mechanical Thermal Analysis) as reported in the literature. These results indicated that the TMCT technique can be applied to measure the glass-rubber transition of the rice in powder form. TMCT technique has the advantage of simplicity and cost-effectiveness compared with the other techniques.

Key words: TMCT, glass-rubber transition temperature, rice, glass transition temperature

INTRODUCTION

Determination of the glass transition temperature (Tg) of rice corresponding to its water content has become very important since the glass transition concept has been applied to explain rice fissure formation during drying (Cnossen, Siebenmorgen et al. 2001; Perdon, Siebenmorgen et al. 2000). However, most of the studies involved in estimating Tg of granular starch has been carried out on the water-starch system (Biliaderis, Page et al. 1986; Chung, Lee et al. 2002; Huang, Chang et al. 1994; Zelenak and Hoseney 1987) rather than native starch or individual kernel. It has been pointed out that DSC (Differential Scanning Calorimetry), a common method to determine glass transition temperature, can not detect the Tg of a low moisture, high molecular weight biological polymers such as starch systems. This is due to several drawbacks related to the nature of native starch at molecular and micro-structural levels and small change in the heat capacity during the transition from one state to other (Biliaderis, Page et al. 1986; Zelenak and Hoseney 1987). For such materials, detection of changes in the mechanical properties has been proved to be more sensitive than the changes in the heat capacity (Roos 1995). Therefore, TMA (Thermo-mechanical Analysis) and DMTA (Dynamic Mechanical Thermal Analysis) are employed to detect the glass transition event of rice kernel (Siebenmorgen, Yang et al. 2004; Sun, Yang et al. 2002). However, in both methods a complex sample preparation technique is required to prevent moisture loss when heating rice sample (Siebenmorgen, Yang et al. 2004).

The recent TMCT (Thermal Mechanical Compression Test) device, which was developed by Bhandari and co-workers at The University of Queensland, Australia, has a potential to
measure the phase transition temperature of any solid materials without much sample preparation step (Bhandari 2007; Boonyai, Bhandari et al. 2006; Boonyai, Howes et al. 2007). This technique is based on the force-deformation or stress-strain response of a material under compression during heating. The amorphous state of material will be transformed from a glassy to rubbery state under the compression and heating, thereby allowing the phase change to be detected by a sudden displacement of the compression probe. The transition is termed as glass-rubber transition ($T_{g-r}$) since the probe displacement occurs due to the change in the viscosity of material at the interface of heating surface. This is similar to a creep test but under thermal scanning condition. The sample cell is designed to be compatible with mechanical analyzers such as Instron or Texture Analyser. Any mechanical changes that occur on the particle surface can be sensitively detected, owing to material being in contact with a large surface area under the compression probe. Furthermore, moisture loss can be minimized since the contact surface of sample is covered by the compression probe. To note, only the change in the mechanical property of the sample surface that is in contact with the heating surface is enough to detect the transition temperature. This TMCT has been applied to detect the glass-rubber transition temperature ($T_{g-r}$) of various dry food materials, i.e. skim milk, whey, honey, and apple juice powders, pasta, and starch powders against standard DSC and TMA methods (Bhandari 2007; Boonyai, Bhandari et al. 2006, Boonyai, Howes et al. 2007). The objective of this work was to investigate the applicability of TMCT to measure glass-rubber transition temperatures of rice flour. The values obtained by this method will be compared with the literature values which are obtained by DSC.

METHODOLOGY

TMCT system. Figure 1 illustrates the TMCT system comprising of a thermally controlled aluminium sample block (50x50x25 cm) which is heated normally at a rate of 30°C/min through heating elements inserted into the sample block. The Texture Analyser TA-XTplus (Stable Microsystems, UK) records the probe position and compression force at specific time, and temperature through 35 mm cylindrical probe and temperature probe, respectively.

In this test, the probe displacement or movement is detected when there is a transformation of physical state of the sample from the glassy to rubbery state. The onset temperature at this point is referred as ‘glass-rubber transition temperature’ ($T_{g-r}$). TMCT is designed to detect glass-rubber transition by heat scanning only. The samples should already be in glass state at ambient condition. It is not possible to measure the reversible transition event while cooling.

Sample preparation

The measurements were done using single kernel of rice or the flour from the same rice sample. Milled YRM64 rice flour was dried in a vacuum oven at 40°C for 24h. The sample was then cooled down in a desiccator and stored in tight container at 25°C for further use. Saturated salts at different water activities were prepared at 25°C as indicated by Bell and Labuza (2000). Aluminum pans each containing a thin layer of 5g dried milled rice flour was transferred into vacuum desiccators. Samples were allowed to equilibrate at 25°C for at least 3 weeks. Moisture content of rice flour was determined according to AOAC method (32.1.02).
Sample data correction and testing procedure

All the tests were carried out under relaxation mode of TA-TXplus (Stable Microsystems, UK). The 35 cylindrical compression probe was used. Pre-dried maltodextrin (DE6) was used as a blank material for data correction (Booyai, Bhandari et al. 2007). Approximately 1g of maltodextrin was spread thinly on the sample cell and held under the probe at the force of 49.033 N for 300 seconds before the thermal scanning. The maltodextrin was then scanned from room temperature to 200°C at a heating rate of 30°C/min. During this thermal scanning, the displacement distances of the probe were recorded. The procedure to conduct TMCT for equilibrated rice flour was similar to maltodextrin, except the scanning temperature up to 150°C was used because rice flour is likely to decompose at higher temperature. Each sample was carried out in triplicate and average value was used as glass-rubber transition temperature. Representative curves obtained during thermal scanning are presented in Figure 2. The changes in the temperature and corresponding probe displacement can be seen in this figure.

Figure 2. The TMCT curve and the temperature line are obtained in TA.XTplus.

Figure 3. Estimation of glass-rubber transition temperature from TMCT curve.
**Determination of glass-rubber transition temperature**

The temperature-distance-temperature data was extracted for further analysis. The corrected curve for each sample was obtained by subtracting the displacement blank data from the sample data. The temperature vs. subtracted displacement was plotted to estimate the $T_{g-r}$ by performing a linear regression as shown in Figure 3.

**RESULTS AND DISCUSSION**

TMCT curves of YRM64 milled rice flour at some selected moisture contents are presented in Figure 4. The slopes were steeper at higher moisture contents indicating that moisture content possibly weakens the intermolecular forces of materials in solid state. Our attempt to measure the transition event using a DSC was not successful. No change could be detected in the DSC thermograms (results not presented). As stated earlier, the sharp changes in the mechanical property of the material allowed detecting the glass transition regions easier than using the DSC technique which is based on specific heat change of material during this transition.

Figure 5 presents the dependence of $T_{g-r}$ on moisture content. It shows clearly that the $T_{g-r}$ decreases with increasing moisture content. This result emphasizes the role of water as strong plasticizer because the presence of water enhances the molecular mobility resulting in lower $T_{g-r}$ according to the free volume theory. The results presented in Figure 5 indicated that the $T_{g-r}$ value was not decreased sharply at higher moisture content, probably due to the limit of the plasticization effect of water on rice. In this case, additional water does not interact strongly with the starch molecules, therefore fails to decrease the $T_{g-r}$ rapidly. The system can behave as phase separated into water and solid.

![Figure 4. TMCT curves of rice flour at some selected moisture contents (wet basis).](image1)

![Figure 5. $T_{g-r}$ as a function of moisture content](image2)

The predicted $T_{g-r}$ in this study was compared to the published data on $T_g$ of rice at the same moisture content range as presented in Table 1. At the moisture content range 12-16% wet basis, predicted $T_{g-r}$ (41.6-56.7°C) was comparable to $T_g$ (approximately 50°C) determined by
DSC (Nehus 1997). At 14.4% wet basis the predicted $T_{g-r}$ in our study was 47.7°C, which was quite close to that of measured by TMA at the same moisture content (Sun, Yang et al. 2002). The $T_g$s defined by DSC and DMTA (around 45°C) at approximately 16% wet basis (Cao, Nishiyama et al. 2004; Sienbenmorgen, Yang et al. 2004) were higher than our measured $T_{g-r}$ (40.38°C). This discrepancy may be explained partly by the evaporation loss in test chamber in DMTA. In DMTA, the measured temperature is not the actual sample temperature but of the test chamber temperature, and the $T_g$ may be higher than the actual value. In addition, the $T_{g-r}$ measured by TMCT is an onset value, whereas in DSC and DMTA analyse it is generally reported as a mid-point value (between $T_{g-on}$ and $T_{g-end}$).

**Table 1.** Published data on $T_g$ of rice as compared to $T_{g-r}$ measured in this study.

<table>
<thead>
<tr>
<th>Moisture content</th>
<th>Samples</th>
<th>Method</th>
<th>$T_g$/$T_{g-r}$ (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-16%</td>
<td>Milled rice</td>
<td>DSC</td>
<td>≈ 50.0°C</td>
<td>(Nehus 1997)</td>
</tr>
<tr>
<td></td>
<td>Rice flour</td>
<td>TMCT</td>
<td>41.6-56.7°C</td>
<td><em>This study</em></td>
</tr>
<tr>
<td>14.4%wb</td>
<td>Brown rice kernel</td>
<td>TMA</td>
<td>45.0°C</td>
<td>(Sun, Yang et al. 2002)</td>
</tr>
<tr>
<td></td>
<td>Rice flour</td>
<td>TMCT</td>
<td>47.7°C</td>
<td><em>This study</em></td>
</tr>
<tr>
<td>16.3%wb</td>
<td>Brown rice kernel</td>
<td>DMTA</td>
<td>45.0°C</td>
<td>(Sienbenmorgen, Yang et al. 2004)</td>
</tr>
<tr>
<td></td>
<td>Brown rice sections*</td>
<td>DSC</td>
<td>45.1°C</td>
<td>(Cao, Nishiyama et al. 2004)</td>
</tr>
<tr>
<td></td>
<td>Rice flour</td>
<td>TMCT</td>
<td>40.38°C</td>
<td><em>This study</em></td>
</tr>
</tbody>
</table>

DSC: differential scanning calorimetry
TMA: thermomechanical analysis
DMTA: dynamic mechanical thermal analysis

*Brown rice sections: brown rice kernel was cross-sectioned into three or four sections.

**CONCLUSION**

In brief, thermal mechanical compression test was found to be applicable to determine the glass-rubber transition temperature of rice flour at a range of moisture content 2.4-19.5% wet basis, which is difficult to detect by common DSC method. The measured $T_{g-r}$ values in this study were not significantly different from those reported using TMA and DMTA techniques. This demonstrates the simplicity and usefulness of this technique to measure the phase transition of not only rice grain but also other cereals or legumes.

**REFERENCES**


Nehus Z., 1997. Milled rice breakage as influenced by environmental conditions, kernel moisture content, and starch thermal properties. Master Thesis. The University of Arkansas, US.


APPLICATION OF CHITOSAN MEMBRANE IN PRESERVATION OF HOA LOC MANGO

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ABSTRACT
Research for appropriate method to preserve Hoa Loc mango is an urgent demand to increase mango export. Application of chitosan membrane in preservation of Hoa Loc mango has shown good results. The quality of mango preserved by chitosan membrane is similar to other methods such as gelatin membrane or CaCl₂ treatment. The combination of using chitosan membrane with other treatments such as using hot water and fungicide can enhance preservative effects.

INTRODUCTION
Vietnam is a tropical country in which many delicious fruits are well-known all over the world. However, the high post-harvest loss of fruit (25%-30%) makes Vietnamese fruit less competitive than others. Therefore, researches on fruit preservation are very urgent in order to reduce post-harvest loss and lengthen their shelf-lives. Hoa Loc mango, one of the most favorite fruits, has 350-600g weight, oval shape, thick pulp, yellow color, nice smell and sweet taste when being ripe.

Chitosan is a biopolymer, a derivative of chitin, extracted from crustacean shell. It is a light, white or light yellow solid without smell and taste. It can dissolve in dilute acid, and melt at 309 – 311°C. Its molecular weight varies from 0.1 to 1.2 million daltons. Among chemicals used in fruit preservation, chitosan is surpassing because it is edible, fungal resistant and biodegradable. Chitosan membrane can limit respiration and ethylene production as well as inhibit the change of fruit color during storage and transportation.

MATERIALS AND METHODOLOGY
Materials
Chitosans: extracted from shrimp-shell by Nha Trang Fisheries University. It has white color, flake shape, molecular weight 1.12 million Dalton, amount of protein and ash less than 1%, and moisture content 10%.

Hoa Loc mangoes: bought at Tam Binh market with same size and mature degree.

Methodology
Method of forming chitosan membrane
Chitosan solution was made by diluting chitosan in 1.5% acetic acid solution. Hoa Loc mangoes were washed, dried and dipped into chitosan solution. They were then dried at room temperature for membrane forming. Treated mangoes were preserved at cool temperature (12-14°C).
Analysis Methodology
- Spoilage rate (%): percentage of infected area over total mango area
- Loss-of-weight rate (%): percentage of differences between initial weight and examined weight over initial weight.
- Firmness (KG/cm²): measured by Penetrometer instrument 13KG
- Brix degree (°Bx): measured by Atago refractometer
- Acid degree: measured by titration with 0.1N NaOH solution
- Color: measured by Minolta Chroma Meter CR–200, L, a*, b* system. Then calculate Degree of color change $\Delta E = (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2$.

RESULT AND DISCUSSION
Effect of degree of deacetylation of chitosan on mango quality

![Graphs showing weight loss, degree of color change, acid degree, and Brix degree for different chitosan concentrations.](image)

Figure 1. Mango quality after 30 days of storage

The appearance of spoilage began only after 20 days of storage on mangoes coated by chitosan 75% DD and 93% DD. All 3 types of chitosan had good effects on reducing weight loss after 30 days of storage (5%). The firmness of all mangoes were still remained during 30 days of storage (>13 KG/cm²). During storage, acid degree reduced while Brix degree increased due to mango ripeness; mango coated by chitosan 85% DD showed less change on these factors. The increase of $\Delta E$ indicated the increase of color-change. Therefore, chitosan 85% DD is more suitable to preserve mango.

Effect of chitosan concentration on mango quality
Figure 2. Mango quality after 30 days of storage

Mangoes coated with 0.5% chitosan showed best results because they had low spoilage rate and weight loss but still remained their firmness, color, acid and Brix degree after 30 days of storage.

Effect of preserving method on mango quality

Figure 3. Mango quality after 42 days of storage

(A: Chitosan; B: CaCl₂; C: Gelatin; D: Polyethylene bag; E: Paper)
Mangoes preserved in polyethylene bag had very high spoilage rate while mangoes preserved by chitosan and gelatin had significant lower ones. Chitosan and gelatin membranes protected mangoes from outside gases and moisture, so they can reduce weight loss during storage. Lower firmness of mangoes preserved in polyethylene bag and paper indicated that they ripened faster than others. Mangoes preserved by chitosan, gelatin and CaCl₂ had higher acid degrees and lower Brix degrees. The results showed that those methods can lengthen the shelf-life of mango.

**Effect of combined treatments on mango quality**

![Graphs showing spoilage rate, weight loss, firmness, degree of color change, acid degree, and Brix degree for different treatments.](image)

**Figure 4.** Mango quality after 42 days of storage  
(A: Chitosan; B: Chitosan + Hot water; C: Chitosan + Benomyl; 
D: Chitosan + Hot water + Benomyl)

Chitosan membrane can be combined with other methods to improve the effects of mango preservation. The combination of chitosan, hot water and Benomyl fungicide can protect mangoes from infection, so their spoilage rate and weight loss were very low. However, mangoes treated with hot water can have higher color-change. This combined method also had high effects on remaining acid and Brix degree and firmness of mangoes. Therefore, the combination of chitosan, hot water and Benomyl fungicide can maintain mango quality during storage.

**CONCLUSIONS**

The application of chitosan membrane in preservation of Hoa Loc mangoes had good effects. The quality of mangoes preserved by chitosan membrane was similar to that of other methods such as gelatin or CaCl₂. The combination of chitosan membrane, hot water and fungicide can be a good method to preserve Hoa Loc mango.
REFERENCES


ASSESSMENT OF PRECOOLING METHODS FOR TURNIPS
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ABSTRACT
In tropical countries, the mean annual temperature usually about 30°C that causes fresh fruits and vegetables rapidly getting spoilage and rot. Therefore, the apply of fast cooling systems is particularly important to maintain the postharvest quality of produce. In this study, turnips were precooled immediately after harvest using vacuum, room and hydrocooling systems to reach 4°C. After that, the produce were stored at 5°C and 90% RH. Half cooling time and quality attributes were measured to assess the performance of all cooling systems.

The shortest cooling time was observed with hydrocooling cooling, followed by vacuum cooling. The half cooling time were 5 minutes and 17 minutes, respectively. On the other hands, room cooling required 35 minutes, two times longer time than vacuum cooling and 7 times longer than hydrocooling to reach half cooling time.

Turnips with vacuum cooling treatment resulted in better maintain of general appearance and longer storage ability (28 days) compared to room cooling (14 days) and hydrocooling treatments (7 days).

INTRODUCTION
Baby white turnips content high nutritional value of complex carbohydrates, K, Mg, a source of vitamins A, B, C and folic acid. They may be eaten raw or as a cooked vegetable and the leaves of turnips are also eaten as “turnip greens”.

Fresh produce starts to deteriorate immediately following harvest. Temperature management is one of the most important factors affecting the quality of fresh produce. Precooling is the first step in good temperature management which can rapidly reduce the field heat from produce prior to storage or refrigerated transport. The cooling rate is dependent on factors: time, temperature, contact and cooling method employed. The recommendation for maximum quality retention of turnips is precooling at 0°C and 95% relative humidity using room, vacuum, hydro and ice cooling.

The study was carried out to estimate cooling time for turnips with room, vacuum and hydrocooling and quality attributes of turnips.

METHODS
Turnips with similar sizes (mean diameter around 3 cm), all harvested in Da Lat – Lam Dong province, were washed, trimmed outer leaves and cut the top to get 5 cm long. After that the turnips were arranged into 4 treatments in a completely randomized block design (RCBD) with 3 replications (2kg/replication).

1. Turnips were hydro-cooled using immersion tank with cool water at 3°C.
2. Turnips were placed in refrigerated room at 3 ± 1°C and 90% RH.
3. Turnips were put in cooling chamber at a low-pressure environment.
4. Control (non- precooling and store at ambient temperature)
The cooling time was stopped when turnip temperature were reduced to 4°C.
Turnip temperature was measured with electronic thermometers calibrated nearest 0,147 - 0,157°C.
After cooling, all treatments were packed in box and kept in a storage room at 5°C with 90% relative humidity to simulate self condition.
During storage, the turnips were evaluated for changes in chemical contents and appearance at days 0, 7, 14, 21, 24 and 28.
Weight loss was determined as percentage of original weight.
Total soluble solids were analyzed using refractometer.
Vitamin C content was determined using titration method with 2,6 – dichlorophenol indophenol.
K content was determined using nuclear activation method.
P content using photometry method.
Ca content using anatomic absorption spectrophotometer.
Changes in visual appearance based on freshness, firmness and color of roots and leaves were evaluated by using a scale of 1-5. A score was considered unacceptable level of lower than 3.
The results were analyzed using Statgraphics version 7.0.

RESULTS AND DISCUSSION
Cooling rate
Cooling time is a parameter that can be used to evaluate the efficiency of fast-cooling systems. Cooling time of all treatments varied with different cooling methods.
Mean initial temperature of turnips was 20±2°C. Hydrocooling is an interesting technology allowing high heat-transfer rates. The temperature could be dropped to 11°C in the half cooling time of 5 minutes. The half cooling time of vacuum cooling system was attained when it reached a temperature of approximately 11,5°C after 17 minutes. On the other hand, room cooling is the slowest way to cool perishable produce. The half cooling time of room cooled turnips was around 35 minutes. Therefore, room cooling treatment required 7 times longer time than hydrocooling to reach 4°C as a result of difference in cooling efficiency.

Water loss
In room and vacuum cooling systems, the field heat is extracted from product as water evaporates that caused room and vacuum cooling treatments lightly reducing in weight. The weight loss of about 0,67% and 1,17% was recorded after cooling, respectively. In hydrocooling system, turnips absorbed water which caused produce 1,50% gain weight. After hydrocooling, the produce were wet conducting risk of disease spread if not be rapidly cooled.
**Nutritional quality**
In general, the quality attributes of turnips such as vitamin C; K, P, Ca contents were not significantly different between 3 precooling treatments during storage.

**Visual appearance**
Turnip leaves which was vacuum-cooled had the best freshness and green color after 24-28 days storage while which was hydro and room-cooled became shriveled and yellowing after 7 and 14 days self life.

Vacuum cooling maintained desirable sensory quality of turnip root during storage. The turnips cooled using vacuum chamber had good appearance and score above the acceptable limit (score of 3) after 24-28 days of storage, respectively, in contrast to that of other precooling treatments. Hydro and room-cooled treatments had visual quality below the acceptability limit after only 7 and 14 days storage. The peel color became milky white with some brown spots and on the surface started to take root.

**CONCLUSION**
Different precooling methods were shown to have differences in their storage ability.
The highest decrease in cooling time was observed with hydrocooling at a temperature of 3°C. However, vacuum treatment resulted in better maintenance of general quality and visual appearance of turnips during 28 days of storage.

**REFERENCES**


AMINO ACID COMPONENTS OF PROTEIN HYDROLYSATE FROM MUSHROOM MYCELIUM (*Agaricus bisporus*)

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ABSTRACT

Because of a high protein content in common mushroom, mushroom have used as a source of protein for human diet for thousands of years. Hydrolysis reaction of protein yield hydrolyzed vegetable protein (HVP) that is a rich of amino acid and peptides mixture. The aim of this study was to investigate and compare the chemical composition, especially amino acid of mushroom protein and its hydrolysate. Mushroom mycelium was acid hydrolyzed, neutralized and spray-dried. Amino acid analysis showed that most of protein in mushroom mycelium was hydrolyzed to free amino acid and short chain peptide. Aspartic acid and glutamic acid were the major amino acids in the hydrolysate and should contribute substantially to enhance flavor.

Key words: amino acid, mushroom, hydrolyzed vegetable protein (HVP)

INTRODUCTION

Due to the increasing cost and limited supplies of animal proteins, and since the vegetable protein is the most abundant source of protein on the earth, a number of vegetable proteins such as peanut and soy bean have been investigated for formulated foods. Among of various source of protein of animal and plant, mycoprotein, which is the generic term for protein-rich foodstuffs made from processed fungus, is an alternative protein source. Not only the common mushroom (*Agaricus bisporus*) has a high protein content (Manning, 1985) but mushroom also contains little fat and digestable carbohydrate, making its suitable for low-calorie diet (Kurtzman, 1997). Therefore, mushrooms have been a part of a normal human diet for thousands of years and, in recent times, amount consumed have risen greatly, involving a large number of species.

The modified form of natural proteins may provide different in their nutrition and functional properties. Protein hydrolyzation is a common food process utilized for the production of flavor enhancers such as hydrolyzed vegetable protein (HVP), hospital diets and protein liquids/powders for weight loss control diets. However, HVP prepared from mushroom protein by acid hydrolysis has not been previously reported. The aim of this study was to investigate and compare the chemical composition, especially amino acid of mushroom protein and its hydrolysate.
MATERIALS AND METHODS

Materials
Mushroom mycelium was cultivated and dried in laboratory-scale (Ministry of Agriculture and Forestry). Waters AccQ tag chemistry package (Amino acid test kits) was obtained from Millipore Corporation (Milford, MA). α-Aminobutyric acid was provided by Acros organics (New Jersey).

Acid hydrolysis
Mushroom mycelium (100 g) was placed in round bottom flask, added 4 N of hydrochloric acid (1 L) under directly flushed with purified nitrogen, and left to hydrolysis at 95 °C for 72 h. After the end of reaction, the pHs of the hydrolysates were adjusted to 6.5 with 10.0 N NaOH, centrifuged and filtered through Celite (0.1 % w/v), spray-dried and stored at 4 °C until analyzed (Jarunrattanasri et al., 2005).

Proximate analysis
Ash, lipid, protein and sodium chloride were determined using standard methods (AOAC 1990). Total carbohydrate was determined by difference.

Degree of hydrolysis (DH)
The DH was defined as the percentage of peptide bonds cleaved (Adler-Nissen 1979) and calculated as number of free amino groups devide by total number of amino acid residues.(Hajós, Mietsch & Halász, 1988).

Amino acids analysis
Samples was analyzed for free amino acids (without hydrolysis) and total amino acids (after hydrolyzed with 6 N HCl at 110 °C for 24 h). The analysis procedure was followed the Waters AccQ tag manual (Millipore Corporation). Calibration was via mixed external Waters amino acid hydrlysate standard. α-Aminobutylic acid was used as internal standard. Reaction of the amino acids with Waters AccQ tag reagent gave derivatives that was detectable by UV light at 254 nm. The relative concentration of individual amino acids were determined by comparing the peak area of each amino acid with that of the calibration curve from α-amiobutylic acid internal standard. All analyses were performed in duplicate and results were repoted as mg/g samples.

Statistical analyses
LSD procedures were used to separated means and difference reported significant at p ≤ 0.05 by Statistix 7 (Analytical software 2000).

RESULTS AND DISCUSSION
Chemical composition of mushroom mycelium and its hydrolysate
Proximate composition of mushroom mycelium and its hydrolysate (M-HVP) in this study were listed in table 1. All of analyzes were very different. The major components of mushroom mycelium were carbohydrate, protein and fat respectively, whereas salt, ash and
protein were the main component in M-HVP. The present of high salt content could be controlled by changing the amount of HCl used or removal by dialysis. 82.98% DH showed that most of protein in mushroom mycelium was hydrolyzed to free amino acid and short chain peptide, which are fast absorb by human. Supported by Ovesen and Allingstrup (1992) used hydrolyzed soybean protein as protein source for weight-losing cancer patients for clinical nutrition.

**Table 1** Composition analysis\(^1\) of mushroom mycelium and its hydrolysate (M-HVP) (dry weight basis) and degree of hydrolysis (DH)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fat</th>
<th>Ash</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Salt</th>
<th>% DH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushroom</td>
<td>14.91(^a)</td>
<td>7.05(^b)</td>
<td>32.46(^a)</td>
<td>44.35(^a)</td>
<td>1.23(^b)</td>
<td>-</td>
</tr>
<tr>
<td>M-HVP</td>
<td>1.00(^b)</td>
<td>12.10(^a)</td>
<td>7.93(^b)</td>
<td>1.53(^b)</td>
<td>77.44(^a)</td>
<td>82.98</td>
</tr>
</tbody>
</table>

\(^1\) Means (n = 3) in the same column with different superscripts are significantly different (p<0.05).

It is generally accepted that measurement of the amino acid content is the most reliable method to determine the protein content (Manning, 1985). The amino acid analyzed of the high protein content (mushroom mycelium) and M-HVP were given in table 2. To compare the eight essential amino acids found that leucine was the predominant amino acids in total amino acids of mushroom mycelium and M-HVP. Aspartic acid and glutamic acid were the major amino acids in the hydrolysate and should contribute substantially to enhance flavor. The over all odor of M-HVP similar to commercial seasoning sauce made from soy bean.

**Table 2** Free and total amino acid compositions (mg/g) of mushroom mycelium and its hydrolysate (M-HVP)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Free amino acids</th>
<th>Total amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mycelium</td>
<td>HVP</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.084</td>
<td>0.107</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.030</td>
<td>0.052</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.078</td>
<td>0.328</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.112</td>
<td>0.190</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.010</td>
<td>0.055</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.022</td>
<td>0.006</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.014</td>
<td>0.051</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.015</td>
<td>0.109</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.014</td>
<td>0.071</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.008</td>
<td>0.005</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.013</td>
<td>0.048</td>
</tr>
<tr>
<td>Proline</td>
<td>0.135</td>
<td>0.109</td>
</tr>
<tr>
<td>Serine</td>
<td>0.044</td>
<td>0.095</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.041</td>
<td>0.077</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.010</td>
<td>0.033</td>
</tr>
<tr>
<td>Valine</td>
<td>0.017</td>
<td>0.058</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.613</td>
<td>1.370</td>
</tr>
</tbody>
</table>
CONCLUSION
Amino acid component and over all odors of sample would conduct protein hydrolysate from mushroom mycelium to be protein source for hospital diets or flavor enhancer for food industry, respectively.

ACKNOWLEDGMENTS
Authors would like to express sincere gratitude to the Korea Science and Engineering Foundation (KOSEF), Republic of Korea for the financial support for this research.

REFERENCES
ABSTRACT

A cross-sectional study was conducted to find the prevalence of porcine cysticercosis in slaughter pigs, sampled from 4 major slaughter slabs in Kathmandu Valley, Nepal for the period of November 2004 to April 2005. In the same period, 8 main hospitals of the valley were surveyed by questionnaires to find the occurrence of human neurocysticercosis. Of the 504 porcine carcasses inspected for cysticercosis, five were found positive, the slaughter prevalence being 0.99% (95% CI: 0.32-2.29). All the sampled carcasses of indoor managed pigs were negative for cysticercosis while the carcasses of both mixed and outdoor-farmed pigs showed the positive results. There was a significant difference in the prevalence of cysticercosis among farming systems (p = 0.001). During 2000 to 2004, neurocysticercosis (NCC) patients were found at the overall rate of 1.02 per 1,000 admission episodes in 6 hospitals. From the remaining two hospitals 1.5 NCC cases were found per 1,000 admission episodes and out patients’ department (OPD) visits. The NCC rate in terms of epileptic admission episodes was 18.7% while in terms of admission episodes and OPD visits of epilepsy, it was 43.2%. Survey data revealed that 32% (25/78) of the NCC cases were from the valley alone. The presence of cysticercosis in slaughter pigs and well-documented data of NCC cases in the hospitals indicate that T. solium, is a real public health threat in Nepal.