Effect of some edible coating films on grapes quality properties during storage
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Abstract
Flame seedless grapes (Vitis vinifera) were coated with four type of edible films the first with 1% chitosan (T1), the second 2% calcium chloride (T2), the thir 1% chitosan, 2% calcium chloride (T3), and the last mixture of 1% chitosan, 2% calcium chloride, 0.2 citric acid, 0.1% ascorbic acids (T4). The samples treate with the edible stored room temperature (28 °C ±3) for 12 days or at cool temperature (4 °C ±1) for 90 days. Shelf-life qualitative weight loss, total soluble solids (Tss), firmness, titratable acidity, sensory evaluation and microbiologici analysis were tested. Significant reduction in weight loss was detected in treated samples compared to control untreated (T0). The treatment T2 was better, th weight loss was 4.6% after 12 day of storage either treatments T4, T3, T1were compared to control (T0) was 7.8% after 4 day of storage. Also, at the cool temperature treatment T2 was less in weight loss it was 4.4% after 90 day compared to control that was 4.7% after 15 days of storage. The better sensor acceptance and acidity retention were detected for the treated samples along the storage period. However, total soluble solids (TSS) varied from (20.1 to 25.23%) at room temperature and (20.2 to 21.83%) at cooling temperature. Fruit firmness were increased where the treatment (T2) was 225 (gm/cm2) compared to the treatment (T1) that was 208 (gm/cm2) at the room temperature (28°C ±3). The treatment (T2) was 209 (gm/cm2) as for the treatment (T1) was 199 (gm/cm2) in the cooling temperature. Also microbiological analysis increases with storage time Total plate count, Psychrophilic bacterial count and molds and yeasts count. Calcium chloride coating (T2) showed best results comparing to those obtained for Chitosan treatments (T1). Coating grapes with chitosan (T1) or calcium chloride (T2) displayed greater external adequacy than untreated ones.

1. Introduction

Because of the beneficial effects on human health and its economic importance, grape is a fruit widely grown and eaten around the world[1].

For over 2,000 years, the grape has been recognized globally as one of the comestible sweet fruits and is known for its broad biological features [2]. The grape fruit has many established nutritional and medicinal properties for consumers and is one of the best foods to be consumed. The grape is a good source of other nutrients like boron [3]. Postharvest deterioration of grapes can be due to physical, physiological, or pathological factors that may take place preharvest or postharvest [4].

Packaging plays a decisive role in the improvement of the shelf life of food products and new packaging materials derived from renewable sources are being developed [5]. The edible coating or film has been defined as a thin, continuous layer of edible substance created or applied on or between food or food components. [6]. Edible films lead to improve food quality since their great advantages in contradiction of non-biodegradable plastic packaging films [7]. Surface coatings reduce breathing and transpiration rates, reduce damage to handling and help maintain structural conditions [8]. Natural polymers of foodstuffs, like polysaccharides and proteins, have received a lot of attention in fruit packaging applications at present [9].

Chitosan is one of the most common natural polymer obtained by deacetylation (β-(1-4)- linked D-glucosamine) of chitin, which is the major constituent of the exoskeleton of crustaceans. It is known to be nontoxic, biodegradable, and biocompatible that can had directly antimicrobial activity [10], [11], [12]. It can improve the functionality of food products (delayed dehydration, suppression of breathing and improve textural quality) and also promote health benefits. In addition to acting as protective barriers of bioactive compounds [13], [14]. Edible coatings made from carbohydrates as chitosan generally exhibits lower moisture barriers [9]. It has been used in agriculture as a coating material for vegetables, fruits and seeds [15], [16], [17].

Calcium (Ca) is the most important mineral element determining fruit quality. It plays a major role in senescence and ripening [18]. Calcium treatments intend increasing calcium content in cell wall, result in to a firmer and higher fruit quality [19], [20] [21].
The aim of this study was to:
1- Evaluate the efficacy of four edible films, chitosan and calcium chloride.
2- Extend the shelf life of the seedless grape variety.
3- Improves the quality of grapes during storage.

2. Materials and Methods
2.1 Plant materials and treatments
Table grapes (Vitis vinifera) of the cultivar flame seedless were harvested at the ripe stage from a private vineyard located at Minia governorate, Egypt. Grapes can’t be harvested when total soluble solids content is below 18%. Fruits were selected for size and color uniformity. Blemished, damaged, or diseased berries were discarded carefully.

2.2 chemicals
Calcium chloride (CaCl₂), chitosan, citric acid, ascorbic acid, glycerol were obtained from (Sigma Chemicals, USA).

Fruits were weighed to 1 kg samples and divided into 5 groups:
( T0 ) - The first group was untreated samples (Control).
( T1 ) - The second group was coated with 1% (w/v) Low molecular weight chitosan.
( T2 ) - The third group was coated with 2% (w/v) calcium chloride.
( T3 ) - The fourth group coated with CaCl₂ at 2%, chitosan at 1%, citric acid at 0.2% and ascorbic acid at 0.1%.
( T4 ) - The fifth group coated with CaCl₂ at 2% and chitosan at 1%.

2.3 Preparation of edible coatings
Edible coatings used in this work were as follows:
coating ( T1 ) - 1%, w/v of chitosan was dissolved in 0.5% (v/v) glacial acetic acid under continuous stirring

Calcium chloride coating ( T2 ) - 2%, w/v of calcium chloride was dissolved in 10% (v/v) glycerol under continuous stirring

Calcium chloride + Chitosan + Citric acid + ascorbic acid ( T3 ) - 1%, w/v of chitosan was dissolved in 0.5% (v/v) glacial acetic acid under continuous stirring .And 2%, w/v of calcium chloride was dissolved in 10% (v/v) glycerol under continuous stirring. Add citric acid at 0.2 % and ascorbic acid at 0.1 %.

Calcium chloride + Chitosan ( T4 ) - 1%, w/v of chitosan was dissolved in 0.5% (v/v) glacial acetic acid under continuous stirring. And 2%, w/v of calcium chloride was dissolved in 10% (v/v) glycerol under continuous stirring. All the treatments ( T1,T2, T3 and T4 ) according to [22], [23], [24], [25], [26], [27].

Sterilized distilled water was used in the preparation of the chemical solutions to prevent contamination.

2.4 Storage conditions
Every treatment was divided into two groups, the first group stored at room temperature (28 °C ±3), and relative humidity in chamber (70±5%) for 12 days. While the second one was stored at cooling temperature (4 °C ±1), and relative humidity in cooling chamber (90±5) for 90 days.

2.5 Analytical methods
2.5.1 Weight loss
Weight loss in grapes stored at room temperature (28 °C ±3) were weighed every 4 days. While samples stored at cooling temperature (4 °C ±1) was determined every 15 days.

Weight loss was expressed as percentage of initial weight the following formula:
\[
\text{Percent weight loss} = \left( \frac{\text{initial weight} - \text{weight at specific interval}}{\text{initial weight}} \right) \times 100
\]

2.5.2 Total soluble solids (TSS)
TSS % was determined by the refractometric method at room temperature using an refractometr (Pocket Refractometer PAL-1) in juice pressed from a sample of homogenized fruit [28].

2.5.3 Firmness of fruit during storage
The firmness of fresh fruit was determined by measuring the compression force of the samples using a Fruits Hardness Tester Cat.Nos. 510-1 (FHR-1).

2.5.4 Fruit titratable acidity
grapes fruit juice samples (10 ml) were used and titrated with 0.1 N sodium hydroxide in the presence of phenolphthalein as an indicator, [29]. The titratable acidity was expressed as % of tartaric acid per 100 ml of juice.

2.5.5 Sensory evaluation
The sensory quality of each replicate berry was evaluated by taste, color, texture, overall acceptability. They were rated on a ten-point hedonic scale (10-7, excellent; 6-4, good; and 3-1, poor); intensity and acceptability increased with the numerical value [27].

2.5.6 Microbiological analysis
The microbiological analysis comprised the determination of total colony count, psychrophilic bacterial count and molds and yeasts was carried out as following :
glass blender jar. The weighed samples were blended for 5 min. The provided a dilution of 10. appropriate serial dilution were made, and then samples were plated by standard microbiological pour plate technique for enumeration [30]. All the microbiological counts were carried out in triplicates

2.5.6.1.1 Total plate count (TPC)

Total plate count of bacteria was determined as (Log CFU/g) using plate count agar medium [30].

2.5.6.1.2 Psychrophilic bacterial count

Psychrophilic bacterial count was determined as (Log CFU/g) described in typical procedure of the total colony count method, except incubation was carried out at 7°C for 5-7 days in refrigerator [31].

2.5.6.1.3 Molds and yeasts count

The mold and yeast were determined using the methods for the microbiological examination of foods described by American public Health association [32].

3. RESULTS AND DISCUSSION

3.1 Effect of coating with edible films on the quality of grapes

3.1.1 Weight loss

Effect of coating and storage temperature on weight loss are showed in fig. (2) and (3). It was obvious that the weight loss was higher in non-coating sample compared to coated ones. As the weight loss in the control sample (T0) reached 7.8% during 4 days and reached 4.62% in the treatment (T2) after 12 days of storage, at room temperature. In cooling temperature, the weight loss in the control sample (T0) was 4.74% after 15 days, and 4.40 % was after 90 days of storage in the treatment (T2). Weight loss is mainly related to the respiration rate and moisture evaporation.

Who suggested that chitosan act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration [33].

The effect of CaCl₂ on weight loss go in line with earlier studies. They realized that the loss in weight during storage of peaches, nectarines, apples and peaches was higher reduced due to preharvest sprays of calcium in the form of calcium chloride or calcium nitrate[34], [35].

3.1.2. Total soluble solid (TSS)

Table (3) and (4) showed the effect of coating treatments on TSS of grapes at room temperature (28 °C ±3) and cooling temperature (4 °C ±1). Comparing to control (T0) and chitosan coating treatment (T1), where the weight loss was in the treatment (T1) it was 7.10% after 12 a day of storage compared to (T2) it was 4.62 in the same time of storage at room temperature. Also it was in (T1) 7.49 % after 90 a day of storage comparison (T2) which was 4.40 % at the cooling temperature (4 °C ±1).

Also storage temperature has a great influence in reducing weight loss rates along the storage period as compared to samples stored at room temperature (28 °C ±3) where the weight loss in treatment T4 was 5.71 % at room temperature (28 °C ±3), but the weight loss reached 0.52 % after 15 day of storage at the cooling temperature (4 °C ±1).

3.1.3. Color

Comparison of color of coated samples to control showed that CaCl₂ treatments had a more effect in reducing the weight loss during storage at both room temperature (28 °C ±3) and cooling temperature (4 °C ±1). Comparing to control (T0) and chitosan coating treatment( T1 ), where the weight loss was in the treatment (T1) it was 7.10% after 12 a day of storage compared to (T2) it was 4.62 in the same time of storage at room temperature. Also it was in (T1) 7.49 % after 90 a day of storage comparison (T2) which was 4.40 % at the cooling temperature (4 °C ±1).

3.1.4. Juice content

Table (3) and (4) showed the effect of coating treatments on juice content of grapes at room temperature (28 °C ±3) and cooling temperature (4 °C ±1) compared to untreated sample (T0) . Juice content increases with the time of storage, as in the treatment (T2) it was at the beginning of storage 22 % and after 12 days of storage it was 23.31% at the room temperature (28 °C ±3). As
well treatment (T4) it was at the beginning of storage 20.7% and after 90 days of storage it was 23.3% at cooling temperature (4ºC ±1). The results are consistent with the other research that the application of chitosan coating limits the respiration rates of grapes, and thus higher levels of total dissolved solids were recorded [36].

Figure 3: Effect of different coating on the weight loss stored at cold temperature (4±1 ºC)

Table 1: postharvest treatments to prolong the shelf life of grapes.

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Control</td>
</tr>
<tr>
<td>T1</td>
<td>1% Chitosan</td>
</tr>
<tr>
<td>T2</td>
<td>2% Calcium chloride</td>
</tr>
<tr>
<td>T3</td>
<td>2% Calcium chloride + 1% Chitosan</td>
</tr>
<tr>
<td>T4</td>
<td>2% Calcium chloride+1% Chitosan +0.2% Citric acid+ 0.1% Ascorbic acid</td>
</tr>
</tbody>
</table>

Table 2: Shelf life of fresh grapes coated with edible films.

<table>
<thead>
<tr>
<th>Emulsion constituents</th>
<th>Shelf life per day at room temperature (28C ±3) (day)</th>
<th>Shelf life per day at under cooling temperature (4C±1) (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>T1</td>
<td>14</td>
<td>95</td>
</tr>
<tr>
<td>T2</td>
<td>15</td>
<td>96</td>
</tr>
<tr>
<td>T3</td>
<td>14</td>
<td>94</td>
</tr>
<tr>
<td>T4</td>
<td>15</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 3: Effect of treatments on TSS of coated grapes at room temperature (28 ºC ±3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage time ( Days )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T0</td>
<td>20.1 ±</td>
</tr>
<tr>
<td>T1</td>
<td>23.4 ±</td>
</tr>
<tr>
<td>T2</td>
<td>22.0 ±</td>
</tr>
<tr>
<td>T3</td>
<td>23.8 ±</td>
</tr>
<tr>
<td>T4</td>
<td>22.2 ±</td>
</tr>
</tbody>
</table>

Table 4. Effect of treatments on TSS of coated grapes at cooling temperature (4 ºC ±1).

<table>
<thead>
<tr>
<th>Treatmen</th>
<th>Storage time ( Days )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T0</td>
<td>20. ±</td>
</tr>
<tr>
<td>T1</td>
<td>20. ±</td>
</tr>
<tr>
<td>T2</td>
<td>21. ±</td>
</tr>
<tr>
<td>T3</td>
<td>20. ±</td>
</tr>
<tr>
<td>T4</td>
<td>20. ±</td>
</tr>
</tbody>
</table>

Values are means, n = 40. Means followed a different letter within row are significantly different according to Duncan’s multiple range test, α = 0.05.

Table 5. Effect of treatments on firmness (lb/inch2) of coated grapes at room temperature (28ºC ±3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage time ( Days )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T0</td>
<td>188 ±</td>
</tr>
<tr>
<td>T1</td>
<td>229 ±</td>
</tr>
<tr>
<td>T2</td>
<td>244 ±</td>
</tr>
</tbody>
</table>

Values are means, n = 40. Means followed a different letter within row are significantly different according to Duncan’s multiple range test, α = 0.05.
Values are treatments means , \( n = 40 \). Means followed a different letter within row are significantly different according to Duncan’s multiple range test, \( \alpha = 0.05 \).

<table>
<thead>
<tr>
<th>T0: Control</th>
<th>T1: 1% Chitosan</th>
<th>T2: 2% Calcium chloride</th>
<th>T3: 2%Calcium chloride+ 1%Chitosan</th>
<th>T4: 2% Calcium chloried+1%Chitosan+0.2% Citric acid+0.1% Ascorbic acid</th>
</tr>
</thead>
</table>

Table 6: Effect of treatments on firmness (gm/cm²) of coated grapes at cooling temperature (4ºC ±1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T0</td>
<td>21</td>
</tr>
<tr>
<td>T1</td>
<td>21</td>
</tr>
<tr>
<td>T2</td>
<td>22</td>
</tr>
<tr>
<td>T3</td>
<td>20</td>
</tr>
<tr>
<td>T4</td>
<td>22</td>
</tr>
</tbody>
</table>

Values are treatments means , \( n = 70 \). Means followed a different letter within row are significantly different according to Duncan’s multiple range test, \( \alpha = 0.05 \)

3.1.4. Fruit titratable acidity

Effect of coating treatments during different storage temperature on titratable acidity of grapes was listed in Table (7) and (8). A reduction in fruit acidity was of grapes noticed in coated samples compared to untreated ones. What is more, chitosan coating treatments showed a better role in maintaining fruit acidity of grapes along storage period as compared to the calcium chloride treatments in both room temperature (28ºC ±3) and at cooling temperature (4ºC ±1). The decrease in fruit acidity during storage period could explained to metabolic changes in fruit so wing to the use of organic acids during fruit respiration, which may give a good indicator that chitosan coating has a greater effect in reducing respiration rates throughout storage period [39].

3.1.5. Sensory evaluation

Effect of different coatings treatments on grapes taste was shown in Fig. 4 and 5. It is obvious that calcium chloride treatment has the highest sensory scores for taste after 12 and 90 days at room temperature (28ºC ±3) and cooling temperature (4ºC ±1), respectively. But all treatments had commercially accepted taste at the end of the storage time. Concerning color, calcium chloride treatment also had the highest impact in both room temperature and cooling temperature storage. But as shown from Fig. 6 and 7 a considerable differences was noticed in color among treatments. The same attitude was noticed for texture among the storage period at room temperature and at cooling temperature storage conditions as noted for Fig. 8 and 9. As for the overall acceptability of all treatments Fig. 10 and 11 at the end of storage, calcium chloride had the higher scores among all panelists which consider a great indicator of what coating should be used in order to satisfy customer’s needs.

Figure 4: Effect of storage period on the taste of grapes at room temperature (28ºC ±3).

Figure 5: Effect of storage period on the taste of grapes at cooling temperature (4ºC ±1).

Figure 6: Effect of storage period on the color of grapes at room temperature (28ºC ±3).

3.1.6. Microbiological analysis

3.1.6.1. Total colony count:

It was observed from Fig. 12 that clear change in the total bacterial count in the grapes coating and un coating during storage at room temperature (28ºC ±3) and by cold (4ºC ±1). Where it was found that the total count of bacteria gradually increased with the increase in the storage period. For example, it was found that total colony count in un coating grapes (T0)
arrived at $8.3 \times 10^2$ (Log CFU/g) after 4 days of storage, as for coating grapes (T3) arrived at $3.33 \times 10^2$ (Log CFU/g) after the same period of storage at room temperature (28°C ±3). Also, it was found that the total number of bacteria gradually increased with the increase of the storage period, as it reached in the treatment (T2) to $9.6 \times 10^2$ (Log CFU/g) after 12 a day of storage compared to the beginning of the storage time was 1.66×102 (Log CFU/g). where found that the coating treatment of fruit and vegetable allowed a limited gases exchange and respiration, moreover, prevent the occurrence of fermentation process and minimized the microbial count.[40], [41], [42]

From Fig. 13 during the storage at cooling temperature (28°C ±3) it was seen that the total number of bacteria for the treatment (T2) reached $7 \times 10^2$ (Log CFU/g) compared to uncoating grapes, arrived $15.33 \times 10^2$ (Log CFU/g) after 30 a day of storage, the treatment (T4) the total count of bacteria was at the beginning of storage 1.33×102 (Log CFU/g) and after 90 a day of storage at cooling temperature it was $16.33 \times 10^2$ (Log CFU/g), that the fruit microbial quality during storage at cooled temperature is better the that occurred at room temperature. [43], [44] and found that coating of apple increased period of storage and delayed ripening depending on sourrounding media in combination with cooled temperature (4°C) and RH (85%) management which exhibit continuity improvement of fruit life. [45], [46]

**Figure 7:** Effect of storage period on the color of grapes at cooling temperature (4°C ±1).

**Figure 8:** Effect of storage period on the texture of grapes at room temperature (28°C ±3).

**Figure 9:** Effect of storage period on the texture of grapes at cooling temperature (4°C ±1).

**Figure 10:** Effect of storage period on the overall acceptability of grapes at room temperature (28°C ±3).

**Figure 11:** Effect of storage period on the overall acceptability of grapes at cooling temperature (4°C ±1).

**Figure 12:** Total colony count of grapes at room temperature (28°C ±3).

**Figure 13:** Total colony count of grapes at cooling temperature (4°C ±1).

### 3.1.6.1.2. Psychrophilic bacterial count:

It was found that psychrophilic bacteria count in coating and uncoating grapes during storage at room temperature (28°C ±3) and cooling temperature (4°C ±1), gradually increases with increasing storage period. Fig. 14, The treatment (T2) It was $1 \times 10^2$ (CFU/g) at the beginning of storage and it arrived at the end of storage after 12 days $4.66 \times 10^2$ (Log CFU/g) at room
temperature (28°C ±3). Also treatment (T3) it was 1 × 102 (Log CFU/g) at the beginning of storage it arrived after 90 days of storage 13.33 × 102 (Log CFU/g) at cooling temperature (4°C ±1) it can be show in Fig.15, found that the increased cooled storage period of fruit may be caused delayed ripening and psychrophilic bacterial counts, depend on surrounding media exhibit continuity improvement of fruit life, in combination with cooled temperature (4°C) and RH (85%) management. [45]

![Figure 14: Psychrophilic bacterial count of grapes at room temperature (28°C ±3).](image)

![Figure 15: Psychrophilic bacterial count of grapes at cooling temperature (4°C ±1).](image)

**Table 7**: Effect of treatments on titratable acidity of coated grapes at room temperature (28°C ±3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T0</td>
<td>0.562</td>
</tr>
<tr>
<td>T1</td>
<td>0.593</td>
</tr>
<tr>
<td>T2</td>
<td>0.562</td>
</tr>
<tr>
<td>T3</td>
<td>0.504</td>
</tr>
<tr>
<td>T4</td>
<td>0.593</td>
</tr>
</tbody>
</table>

Values are treatments means, n = 40. Means followed a different letter within row are significantly different according to Duncan’s multiple range test, α = 0.05

**T0**: Control  **T1**: 1% Chitosan  **T2**: 2% Calcium chloride  **T3**: 2% Calcium chloride+1% Chitosan  **T4**: 2% Calcium chloride+1% Chitosan +0.2% Citric acid+0.1% Ascorbic acid

3.1.6.1.3. molds and yeasts count:

The results were shown in Fig. 16 and 17 the mold and yeast counts in the coating and un coating grapes gradually increases during storage at room temperature and cooling. It was 0.33 × 101 (Log CFU/g) in the treatment (T1) at the beginning of storage and reached to 9 × 101 (Log CFU/g) after 90 days of storage at a cooling temperature. while the treatment (T3) was 0.33 × 101 (Log CFU/g) at the beginning of storage and it reached to 3.66 × 101 (Log CFU/g) after 12 days of storage at room temperature. the results indicated that the mold and yeast counts gradually increased with increasing of storage period at room and cooled temperature in both foam tray and carton boxes so This is may be due to the increasing of RH in refrigerating chamber and suitability of the refrigerator temperature for yeast growth according to [47].

![Figure 16: molds and yeasts count of grapes at room temperature (28°C ±3).](image)

![Figure 17: molds and yeasts count of grapes at cooling temperature (4°C ±1).](image)
4. Conclusion:
Calcium chloride coating treatment (T2) had considerable impact in maintaining grape quality during storage in both room temperature (28°C ±3) and at cool temperature (4°C ±1). It was observable that chitosan coating treatment (T1) had a higher effect in reducing changes in fruit acidity among storage period.

5. Acknowledgment
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[27] Po-Jung Chien ‘Fu Shu Shue, Feng-Hsu Yang (2005). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. A Department of Food Science, Yuanpei University of Science and Technology, No. 306, Yuanpei Street, 300, Hsinchu 30051, Taiwan, ROC b Department of Horticulture, College of Agriculture and Biore sources, National Taiwan University, Taipei 10673, Taiwan, ROC Received 23 March 2005; accepted 26 September 2005.


