Numerization of Peeling Easiness and Role of Phenolic Compounds of the Pellicle in the Adhesion between the Pellicle and Embryo in Comparison of Japanese (Castanea crenata Sieb. et Zucc.) and Chinese (Castanea mollissima Blume) Chestnuts

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Summary
The degree of peeling easiness, i.e., easiness to remove the pellicle from the kernel in roasted nuts, was numerized using "peeling score". Peeling score was obtained by grading the peeling easiness of roasted nuts into four grades, based on the peeling time, the time required to peel off the pellicle from kernel with a knife without damaging embryo. The peeling score correlated to the peeling time of raw nuts ($r=0.78^{**}$), and also to the strength of adhesive force between the pellicle and embryo of raw nuts evaluated by a rheometer ($r=0.81^{**}$). The peeling score seemed to be useful to evaluate briefly the easiness of peeling off the pellicle in chestnuts, and to improve the efficiency of selection in chestnut breeding.

The pellicle of Japanese chestnut was completely stripped by incubating the nut in sodium chlorite solution, which was known to solubilize phenolic compounds, without visible damage to the surface tissues and pleats of the embryo. This fact suggested that the phenolic compounds of the pellicle played an important role in the adhesion between the pellicle and embryo.

Free and polymerized phenolic compounds in the pellicle were determined separately by precipitating the latter with gelatin. In Japanese chestnuts, which are difficult to peel off their pellicles, the amount of total phenolic compounds in pellicles increased rapidly with their maturation, and the increments were mainly composed of polymerized ones. The change in the amount of phenolic compounds with developmental stages of nuts corresponded to that of the peeling time of the pellicle from kernels, and the positive correlation of $0.89^{**}$ was obtained between the amount of phenolic compounds and the peeling time in raw nuts. In Chinese chestnuts, whose pellicles are easily removed from kernels, the amount of total phenolic compounds contained in the pellicles of matured nuts was less than 50% of that of matured Japanese chestnuts. The increase of polymerized phenolic compounds with nut maturation was also less than that of Japanese chestnuts.

These results suggested that the rapid increase of phenolic compounds, especially polymerized ones, with nut maturation might be involved in the relative easiness of peeling off the pellicles of chestnuts.

Introduction
The chestnut is one of the popular nuts and is utilized by roasting and boiling, or is processed into sirup pack and marrons glacés. In the utilization of Japanese chestnuts (Castanea crenata Sieb. et Zucc.), the most troublesome problem is to peel off the pellicle, because of hard adhesion of the pellicle to the embryo. Now, this peeling is performed by using a knife in home use and also in com-
To peel easily, some studies have been done from physical or chemical viewpoints. Recently, the new machine was devised by Kikkoman Shoyu Co. (Pat. No. 46-40748, 1972). This machine has an ability to peel off the pellicle together with the shell using high temperature and high pressure. However, there seem to be some problems to be overcome for further practical applications. Manabe (8) tried to remove the pellicle with hydrogen chloride, with nitric acid and with perchloric acid, but could not succeed in practical applications.

On the other hand, some breeders have pointed out a possibility that new cultivars, which can be easily peeled off, might be bred by introducing the character of Chinese chestnuts (Castanea mollissima Blume), whose pellicle is easily peeled off, into Japanese chestnuts. Thus, hybridization between Japanese and Chinese chestnuts was experimented. And the pellicles of some hybrids were reported to be removed easily (12). To improve the selection efficiency in the breeding by such hybridization, it is necessary to numerize the strength of adhesive force between the pellicle and embryo in roasted chestnuts. For that purpose, the inheritance and mechanism of adhesion between the pellicle and embryo must be studied in detail by making clear the chemical or physical characters of pellicles in comparison of Japanese and Chinese chestnuts.

It is well known that the pellicle of chestnut is rich in tannin, but a few reports mentioned about phenolic components of the pellicle (1, 3, 5). In general, tannin interacts with protein (2, 7, 10) or polysaccharide (9, 13), and is known to form complexes. In the chestnut pellicle, also, such interaction may be functional for the adhesion of pellicle.

In this paper, we intend firstly to numerize the adhesive force between the pellicle and embryo, and secondly to clarify the relation of phenolic components to the adhesive force in comparison of Japanese and Chinese chestnuts and their hybrids.

**Materials and Methods**

**Materials:** Two species of chestnuts and their hybrids were used in the experiment. One is Japanese chestnut (Castanea crenata Sieb. et Zucc. cvs. ‘Tanzawa’, ‘Tsukuba’ and ‘Ishizuchi’), whose pellicle is difficult to peel off. Female flowers of ‘Tanzawa’ and ‘Ishizuchi’, and those of ‘Tsukuba’ were pollinated on Jun. 15, 1979 with pollens of ‘Tsukuba’ and ‘Tanzawa’, respectively. These trees were cultivated in the orchard of Ibaraki Ken Horticultural Experiment Station. Nuts were harvested several times over the following periods, i.e. from Aug. 2 to Aug. 29 in ‘Tanzawa’, from Aug. 17 to Sep. 27 in ‘Tsukuba’ and from Aug. 30 to Oct. 11 in ‘Ishizuchi’.

The other is Chinese chestnut (Castanea mollissima Blume). The cultivars used were ‘Konansho-39’ and ‘Konansho-50’ (selection numbers), open pollinated seedlings developed from nuts which were collected at several provinces in People’s Republic of China and their pellicles were peeled off easily. Their flowers were pollinated in our orchard on June 21, 1979 with pollens of ‘Konansho-38’. The nuts were harvested at several times from Sep. 20 to Oct. 20, 1979.

The open-pollinated nuts of hybrid-trees (Japanese chestnut(J)× Chinese chestnut (C), C×J, F1×C and F1×F1) in our orchard were collected at their matured stages.

**Peeling of the pellicle from the kernel:** Harvested nuts were roasted with a commercial “Yakiguriki” (chestnut roaster containing heated small stones) for 30 min by adding sirup sometimes. Then, the peeling time of the roasted nuts was measured. In this paper, the peeling time of roasted or raw chestnuts was defined as the time required to peel off the pellicle from the kernel without damaging embryo, after removing the nut shell. Roasted nuts were divided into four grades according to the peeling time. The grades of 0 to 3 indicated that the pellicle was peeled off, together with the shell spontaneously (0), within one min (1), within 1 to 5 min (2) and beyond 5 min (3), respectively. Then, the peeling score was calculated as follows and used to express the
relative easiness of peeling in each cultivar: The peeling score = \( \frac{0 \times N_0 + 1 \times N_1 + 2 \times N_2 + 3 \times N_3}{N_0 + N_1 + N_2 + N_3} \)

The numbers of nuts belonging to the grades 0, 1, 2, and 3, respectively. In most cases, 20 nuts were used for each cultivar.

**Measurement of adhesive force between the pellicle and the embryo:** After removing the shell from nut and scraping fiber tissues carefully from the surface of the pellicle, a section of 1 cm cube with pellicle was cut out from the central part of the nut. The pellicle side of the cube was grooved circularly (\( \phi 5 \text{ mm} \)) to the depth of the pellicle itself with a cork-borer. A wooden piece (\( \phi 5 \text{ mm} \)) was adhered vertically to the above circularly grooved area using a chemical bond (Hi-quick, from Cemedain K.K.). After standing the cube for 2 hr, both the cube and the edge of wooden piece were fixed to a rheometer (Fudo Co. Ltd. Model RUD-J). Then, the cube was tensed till the pellicle was separated from the kernel. The force for separation was measured. The average of ten measurements was obtained in each cultivar.

**Removing of the pellicle by sodium chlorite:** The shells of nuts were removed, and these nuts were reacted in about 10 ml of incubation medium containing 0.25g sodium chlorite and a few drops of acetic acid per g nut fresh weight, and kept at 60°C for 10 hr.

**Extraction and determination of phenolic compounds:** Fresh pellicles were homogenized in 80% methanol, and the homogenate was centrifuged at 7000 g for 10 min. The precipitate was washed twice with 80% methanol. The three extracts were combined and used for analysis of phenolic compounds. The amount of total phenolic compounds was determined by the absorbance at 700 nm by employing (+)-catechin as standard, according to the method of Folin–Denis.

**Separation of free and polymerized phenolic compounds:** Free phenolic compounds were separated from polymerized ones by precipitating the latter with gelatin, according to the modification of Nakabayashi’s method (11). To precipitate polymerized phenolic compounds, 1 ml of 4.5% gelatin solution saturated with sodium chloride, 2 ml of 0.9 N sulfuric acid saturated with sodium chloride and 0.5 g of Kaolin were added to 2 ml of 80% methanol extract. The mixture was vigorously shaken for 5 min, and then centrifuged at 3000 g for 10 min. The supernatant was used for the analysis of free phenolic compounds, according to the method of Folin–Denis.

**Thin layer chromatography (TLC) of phenolic compounds:** Phenolic compounds were separated by TLC (Merck TLC plates Silica gel 60) employing n-butanol : acetic acid : water (2 : 2 : 1 v/v) as a developing solvent. The separated spots were detected by the absorbance at 280 nm using a chromatoscaner (Shimadzu Co. Model CS-910).

**Determination of moisture content of the pellicle:** The moisture contents of pellicles were obtained by drying them for 2 hr at 130°C.

**Results and Discussion**

**Numerical evaluation of easiness of peeling off the pellicle by application of peeling score:** It is well known that Japanese chestnuts are very hard to peel off the pellicle from the kernel, while Chinese chestnuts are very easy to peel. In the breeding of chestnuts, a simple method has been urgently required to estimate numerically the degree of easiness of peeling in roasted nuts, but it has not yet been established. In this paper, the peeling easiness of roasted nuts was classified into 4 grades according to the peeling time and the peeling score of each cultivar was obtained as described above.

Firstly, it was checked whether the peeling score of roasted nuts reflected directly the peeling easiness in raw nuts. As shown in Fig. 1, which was obtained with 11 hybrids between Japanese and Chinese chestnuts, the peeling score of roasted nuts was shown to correlate closely with the peeling time of raw nuts (\( r=0.78 \) significant at 1% level, \( Y=14.8 X+0.58 \)).

The peeling easiness of pellicles in roasted nuts seemed to correlate to the strength of adhesive force between the pellicle and embryo of raw nuts, which, then, was measured by the stretching experiment with a rheometer, and compared with the peeling score of roasted...
nuts. The adhesive force was within the range 0 to 5 kg/cm², and it also correlated closely with the peeling score of roasted nuts (r = 0.81 significant at 1% level), as shown in Fig. 2. The weaker the adhesive force in raw nuts, the easier the peeling off the pellicle in roasted nuts. Thus, the peeling score proved to be useful to evaluate briefly the easiness of peeling off the pellicle of roasted nuts.

**Peeling score varying with cultivars:** Peeling score was highest in Japanese cultivars such as ‘Ginyose’ and ‘Tsukuba’, while lowest in Chinese cultivars. Peeling scores of the hybrids were distributed broadly between those of Japanese and Chinese cultivars. Among the hybrids, seedling numbers 345-1 [68-13 (Hoji-360 (C) × Toyotamawase (J)) self] and 344-17 [68-13 × Hoji-460 (C)] showed low peeling scores (0.0 to 0.3 and 0.05) comparable to that (0.1) of Chinese cultivar. So, it is possible to select hybrids whose pellicles are

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**Table 1. The numerical evaluation of peeling easiness of roasted chestnuts**

<table>
<thead>
<tr>
<th>Cultivar or selected seedling</th>
<th>Harvesting season</th>
<th>Cross combination†</th>
<th>Average weight (kg)</th>
<th>Number of nuts examined</th>
<th>Grade of peeling easiness‡</th>
<th>Peeling score‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese chestnut</td>
<td>Collected in People’s Republic of China</td>
<td>6.9</td>
<td>20</td>
<td>18</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Japanese chestnut</td>
<td>Ginyose</td>
<td>1977</td>
<td>20.4</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Tukuba</td>
<td>1977</td>
<td>17.5</td>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Hybrids</td>
<td>344-17</td>
<td>68-13 (F) × Hoji-480 (C)</td>
<td>13.0</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>345-1</td>
<td>68-13 (F) self</td>
<td>9.4</td>
<td>20</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>347-17</td>
<td>308-29</td>
<td>Shimakii-6 (F) × MU-9 (F)</td>
<td>8.9</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>360-19</td>
<td>Toyotamawase (J) × Miyagawa-85 (C)</td>
<td>6.4</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1978</td>
<td></td>
<td>13.1</td>
<td>6</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>349-26</td>
<td>363-9</td>
<td>NA-9 (F) × Aioi (C)</td>
<td>9.5</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>308-11</td>
<td>Otomune (J) × Hoji-480 (C)</td>
<td>14.0</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>350-18</td>
<td>Shimakii-6 (F) × MU-9 (F)</td>
<td>14.2</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>360-3</td>
<td>Hayashimaguri (F) × Aioi (C)</td>
<td>9.7</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1978</td>
<td>356-16</td>
<td>Toyotamawase (J) × Miyagawa-85 (C)</td>
<td>9.5</td>
<td>20</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>1978</td>
<td>Toyotamawase (J) × Hoji-480 (C)</td>
<td>10.7</td>
<td>20</td>
<td>2</td>
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<td></td>
<td></td>
<td>356-16</td>
<td>Toyotamawase (J) × Miyagawa-85 (C)</td>
<td>11.2</td>
<td>20</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>1978</td>
<td>Toyotamawase (J) × Miyagawa-85 (C)</td>
<td>10.8</td>
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<td>—</td>
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<tr>
<td></td>
<td></td>
<td>1978</td>
<td>Toyotamawase (J) × Miyagawa-85 (C)</td>
<td>12.6</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

†C, J and F express Chinese chestnut, Japanese chestnut and their hybrids, respectively.
68-13 [Hoji-360 (C) × Toyotamawase(J)], MU-9 [Hoji-480 (C) × Ganne(J)], NA-9 [Katayama(J) × Hoji-480 (C)]
‡Rating is based on 0: the pellicle is peeled off spontaneously with the shell and 1 to 3: the pellicle can be peeled off within 1 min, 1 to 5 min and beyond 5 min, respectively.
Peeling easiness varying with maturity: The peeling time of raw nuts was measured from the immature to mature stages to certify when the pellicle of Japanese chestnuts became more difficult to peel off. As shown in Fig. 3, the pellicles of Japanese cultivars ‘Tanzawa’, ‘Tsukuba’ and ‘Ishizuchi’, were easily peeled off in the early developmental stages, i.e. till Aug. 17, Aug. 30 and Sep. 20, respectively. But they became abruptly difficult to peel off, and required longer peeling times, with nut maturation. Such difficulty in peeling off the pellicle was further intensified in the nuts which had fallen out of the bur after maturation, and it seemed to be due to the rapid dehydration of the pellicle (see Fig.8). In the case of Chinese cultivars ‘Konansho-39’ and ‘Konansho-50’, however, the pellicles were easily peeled off over all developmental stages and also after drop. It was suggested that the rapid chemical changes in pellicle components occurred at the maturation stage of Japanese chestnuts.

Relationship between peeling easiness and phenolic compounds of the pellicle: In general, the pellicle is rich in tannins. The tannins are known not only to polymerize themselves, but also to form the complexes with polysaccharides and proteins. So, the chemical changes of phenolic compounds in the pellicle were investigated.

1. Removing of the pellicle with sodium chlorite: In lignificated woody tissues, it had been reported that lignin and other phenolic compounds were decomposed oxidatively by sodium chlorite and solubilized into water(4, 6). The pellicles of chestnuts are known to be rich in phenolic compounds, and when raw nuts are easy to peel off, by applying the peeling score.
were incubated in a solution of sodium chlorite for 10 hr at 60°C, the pellicles were completely stripped off as shown in Fig. 4, while were not without sodium chlorite. The surface of the embryo after stripping off the pellicle was not damaged, at least, visibly and the pleats on the surface was kept intact. The fragments of colorless pellicles were released into the incubation medium possibly because their phenolic compounds had been solubilized into the medium. These facts suggested that the phenolic compounds of the pellicle might play an important role in the adhesion between the pellicle and embryo.

2. Separation of free phenolic compounds from polymerized ones by gelatin method: To search the role of phenolic compounds in adhesion between the pellicle and embryo, the changes with the developmental stages of the free and polymerized phenolic compounds contained in the pellicle were estimated in comparison of Japanese cultivars, ‘Tanzawa’, ‘Tsukuba’, and ‘Ishizuchi’ and Chinese cultivars, ‘Konansho–39’ and ‘Konansho–50’. At first, it is necessary to separate the free phenolic compounds from polymerized ones. Figure 5 shows the final concentration of gelatin required to precipitate polymerized phenolic compounds. In the case of ‘Konansho–39’ containing the least total phenolic compounds among the five cultivars used (see Fig. 6), polymerized phenolic compounds were almost perfectly precipitated by adding 0.4% of gelatin (final concentration) and the precipitate increased no more by adding more than 0.4% of gelatin. In the case of ‘Tsukuba’ containing the most total phenolic compounds (see Fig. 6), polymerized phenolic compounds were almost perfectly precipitated by adding 0.8% of gelatin. On the other hand, free phenolic compound

Fig. 5. The separation of free phenolic compounds from polymerized ones by gelatin method.

Fig. 6. Changes with the developmental stages in the amounts of total, free and polymerized phenolic compounds contained in pellicles on fresh weight basis. ●, ○, ●, ○: the nuts which had fallen out of the bur after maturation.
[(+)-catechin] was hardly precipitated, even if the gelatin concentration was elevated to 1.4%. So, 0.9% gelatin (final concentration) was adopted as a standard procedure to separate free phenolic compounds completely from polymerized ones.

3. Free and polymerized phenolic compounds varying with developmental stages of nuts:
In Japanese chestnuts, the content of total phenolic compounds of pellicles on fresh weight basis increased rapidly with maturation (Fig. 6). The increased phenolic compounds were mainly composed of polymerized ones. Free compounds were present in small amounts and kept almost constant over all the developmental stages. Such findings were also supported by the TLC profiles of total phenolic compounds shown in Fig. 7, which indicated that polymerized phenolic compounds located in the lower Rf value increased clearly with maturation (Sep. 13 and Sep. 20), while free phenolic compounds standardized with (+)-catechin (Rf value of 0.9) were detected scarcely during immature stages. Furthermore, the changes with the developmental stages in the contents of total and polymerized phenolic compounds (Fig. 6) corresponded to those of the peeling time of the pellicles (see Fig. 3, \(r=0.89\) significant at 1% level). While, in Chinese chestnuts, the content of total phenolic compounds, most of which were present in polymerized forms, was less than 50% of that of matured Japanese chestnuts, and increased little with maturation. Free phenolic compounds were kept at a low level over all the developmental stages as in Japanese chestnuts.

The distinctive increase in the content of phenolic compounds of both Japanese and Chinese chestnuts after falling out of the bur following maturation seemed to be caused by the dehydration of pellicles, and not by the increase of phenolic compounds per se. After falling out of the bur, the moisture percentages of pellicles decreased rapidly to about 20% on fresh weight basis, although they were kept constantly at about 60% before harvest (Fig. 8). While the contents of phenolic compounds on dry weight basis rather decreased after dropping in both Japanese and Chinese chestnuts cultivars, although they increased abruptly just before dropping only in the Japanese cultivars like those in fresh weight basis (Fig. 9).

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Fig. 7. TLC chromatogram of phenolic compounds contained in the pellicle of Japanese chestnut, cv. 'Tsukuba'.
The arrow indicates the spot of (+)-catechin.
*: the nuts which had fallen out of the bur after maturation.

Fig. 8. Changes with the developmental stages in the moisture content of pellicles. ○, ●, ■, ▲: the nuts which had fallen out of the bur after maturation.
From these results, it was suggested that the rapid increase in phenolic compounds, especially polymerized ones, led to make it difficult to peel off the pellicles from kernels with nut maturation. In general, tannins interact with proteins to form complexes (2, 7). Morrawiecki (10) reported that tannins bound to protein by forming multiple hydrogen bonds between their phenolic hydroxy groups and the carbony groups of the peptide linkages of proteins. And lignin, a kind of phenolic compounds, was reported to form complexes with polysaccharides (9, 13). So, in chestnuts it may be possible that the polymerized phenolic compounds accumulate in the space between the pellicle and embryo, and form complexes with proteins or polysaccharides contained in the cell wall of the surface of pellicle and embryo, and resultantly the formation of their complexes makes it difficult to peel off the pellicle. In the future, it will be necessary to clarify the mechanism of binding between the pellicle and embryo by searching the structures and characteristics of monomers constructing polymerized phenolic compounds and the process of polymerization.

Acknowledgements

The authors express their sincere thanks to Dr. I. Shimura at the Tokyo University of Agriculture and Technology and Dr. S. Yamaki at the Fruit Tree Research Station for their valuable discussions and kind help in the preparation of the manuscript, and also to Ibarakiken Horticultural Experiment Station and all the students in the college of our research station for their assistance in the experiments.

Literature Cited

9. MARKWALDER, H. U. and H. NEUKOM. 1976. Diferulic acid as a possible crosslink in hemi-
クリの剥皮とはいの接着力の数値化と，接着におけるフェノール成分の役割

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摘 要

クリの利用加工上の最大の難点は剥皮の刷（はく）皮の困難さであり，当場育種グループは，刷皮性の良い優良品種の育成を行ってきた。本報では剥皮の刷皮の難易度の数値化と，剥皮の難易におけるフェノール成分の役割を検討した。

焼きクリの刷皮の難易度を peeling score を用いることによって数値化した。peeling score は，はいを傷つけることなく，ナイフで剥皮を剥皮するに要する時間によって4段階に区分して求めた。焼きクリの peeling score は剥皮の難易度と正の相関（γ=0.78**)を示し，かつ，レオノメーターによって測定された生クリでの剥皮とはいの接着力の強さとも正の相関（γ=0.81**）を示した。この数値化はクリにおける剥皮刷皮の難易度を簡単に評価することができ，クリの育種選抜の効率化に役立つものと思われる。

剥皮を除いたニホングリを亜塩素酸ソーダで処理すると，果肉表面を傷つけることなく，剥皮を完全に除去でき，この事実は剥皮に含まれるフェノール成分が剥皮とはいの接着に重要な役割をもっていることを示唆した。

剥皮中に含まれる遊離型フェノールと重合型フェノールを，後者をゼラチンによって沈殿除去することによつて，分別定量した。剥皮の剥けにくいニホングリで，未熟から成熟まで剥皮中のフェノール含量を時的に測定したところ，フェノール含量が果実の成熟に伴って急激に増加し，この増加は主に重合型フェノールに依存した。このフェノール量と剥皮刷皮時間の経時変化の傾向は一致し，フェノール量と剥皮刷皮時間の間には正の相関（γ=0.89**)があった。剥皮が剥けやすいチェウォクグリでは，フェノール量は少なく，ニホングリの1/2以下であり，重合型フェノールの増加も少なかった。

これらの結果から，剥皮中に含まれるフェノール成分，特に重合型フェノールの蓄積がクリの剥皮刷皮を困難にする原因の一つであると考えられた。