

Effects of repeated short-term skin contact with proteolytic enzymes

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The objective was to investigate effects on the hand of protease used in dishwashing liquids (DWL). A group of 30 Koreans (normal skin) and 24 Japanese (12 atopic and 12 normal) were employed for two different studies, respectively. The 30 Koreans were divided into three groups. Each group immersed their hands for 15 min a day for 9 days into DWL containing 0.005% protease, 0.02% protease, or non-enzyme DWL. The 24 Japanese immersed their hands for 15 min a day for 4 days into 0.005% protease-containing or a non-enzyme DWL. The hand skin was evaluated by measuring overall and dry skin grades, estimated before and after exposures to the test products. The Korean study shows that 0.005% and 0.02% protease-containing DWL are less irritating to the skin than non-enzyme control, as measured by better overall skin grades and less dry skin. The Japanese study shows that 0.005% protease-containing DWL are less irritating to the skin than non-enzyme control in both atopic and normal subjects. The 0.005% protease use did not cause any adverse dermatological effects to atopic subjects compared with non-enzyme control. Both studies indicate that 0.005% and 0.02% protease resulted in better skin mildness profile, without causing adverse dermatological complications to the subjects.

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Enzymes in detergents help enhance cleaning efficacy by breaking water-insoluble compounds into smaller, water-soluble fragments at a broad range of water temperatures. The first known commercial enzyme use in detergent products was in 1913 (1). By the late 1960s and early 1970s, 75% of laundry detergent products contained enzymes (2).

Consumers' habits and practices in several countries with enzyme-containing laundry detergent products are to use the products for hand laundry, dishwashing, or personal cleansing, which causes direct exposure of the skin to enzymes. High concentration enzyme exposure may cause dermatological complications. Several studies (3–5) have also recently reported the skin irritation capability of proteolytic and lipolytic enzymes. Conversely, other dermatological and toxicological evaluation studies have detected no adverse effect of enzyme-containing detergents (1, 6–8). Andersen et al. (8) did not detect any significant irritant capacity of detergent enzyme consisting of protease at 1%, lipase at 0.4%, and amylase at 0.4% in atopic der-

matitis patients. These diverging results may be caused by the concentration of active enzymes, the frequency of exposures to enzyme, length of exposure, variations in test techniques, and varying criteria for reading skin reactions, which was suggested by Belin & Norman (9).

To ensure an acceptable skin safety profile of enzyme-containing DWL, two direct application hand immersion tests (DAHIT) were conducted with 30 normal Korean subjects for 2 weeks and with 24 Japanese subjects (12 normal and 12 atopic) for 1 week. The DAHIT study protocol was designed to simulate real dishwashing habits and practices of Korean and Japanese consumers who use a sponge and directly apply DWL on it. This is referred to as 'Direct Application Habit'.

Materials and Methods

Materials

Three types of dishwashing solution samples were provided by the Procter & Gamble Company. The

matrix of each DWL sample contained 0.005% protease, 0.02% protease, or non-protease. The protease used was a commercial raw material that was a typical of those currently used in laundry products. Except for the presence of protease, the DWL formula was a conventional household dishwashing formulation currently sold on the market. Binocular microscope (7 $\times$) (Meiji EMZ-TR[®], Meiji Techno. Co., Japan) with a lighting system was used to evaluate the overall hand skin status and its dryness.

Panel volunteers

The criteria for panelist selection were as follows:

- at least 18 years of age;
- free of active dermatitis on the hands at the initial examination or not exhibiting other dermatological conditions that, in the opinion of the clinical examiner, could compromise test integrity or make overall skin grading unduly difficult (e.g. extreme hyperhydrosis);
- normally wash dishes by hand each day using Direct Application Habit;
- agreement not to use lotion on their hands during the course of the study;
- no participation in skin studies 60 days prior to this test.

Based on the clinical examiner's examination of each panelist's hand-skin conditions, the Japanese panelists were divided into the 12 normal and 12 atopic groups. Atopic subjects were selected based on the medical history and results of the skin prick test (SPT) (9, 12). The SPT was performed by placing a small drop of each test antigen and control solution on the anterior surface of the forearm. The allergens used for the SPT were dermatophagoides farinae, Japanese cedar, grass pollen mixture and *Candida albicans*, allergens that are common in Japan. The control and antigen solutions were placed with a blunt tip large gauge needle on the end of a small syringe. A separate placement instrument was used for each control and antigen solution to prevent cross-contamination and false positive reactions. Results were read 15 min after pricking the skin. A positive skin prick test reaction is a wheal, 3 mm or greater than the negative control, with a flare (bigger than the wheal). If a reaction met these criteria, the skin prick testing was immediately repeated on the other arm to confirm the positive reactions. A subject was considered sensitized only when the initial and confirmation skin pricks induced a positive response to the test antigen. One Japanese panelist did not have atopic history, but showed a positive allergic reaction in the SPT and was regarded as atopic. All Japanese subjects were females between

20 and 55 years old. All Korean panelists employed were female subjects who reported normal skin. No SPT was performed on these subjects. The age range of Korean subjects was between 22 and 40 years. The Korean study was conducted in late April/early May, while the Japanese study was conducted in early March. The temperature in early March in Osaka, Japan, was 5–10°C, while the temperature in late April/early May in Seoul, Korea, was 15–23°C.

Exposure conditions

All panelists were instructed to wear rubber gloves during the study when they engaged in non-dishwashing or cleaning tasks. The panelists were also reminded to refrain from using a lotion on hands during the study. Exposure conditions to DWL for the test were based on the consumers' habits and practice of using DWL in Korea and Japan. The Direct Application Hand Immersion Test protocol was developed by the Procter & Gamble Company to simulate the dishwashing habits and practice in Korea and Japan. The procedures for the exposure to DWL were as follows:

1. 2 L of tap water (20°C) was placed in a bucket.
2. A sponge was placed in water and squeezed gently to remove excess water and then placed on countertop.
3. 5 mL of DWL was evenly applied to one side of the sponge.
4. The panelist put both their hands into the bucket to wet them and then took their hands out. The panelist squeezed the two sponges containing DWL with different levels of protease with his/her left and right hand, respectively. One side of the sponge with 5 mL of the product was applied directly to the panelist's palm for 5 seconds, which was enough time to lather the palm and between the fingers with some dorsal coverage. The panelist then held each sponge with the lather on hands for the remaining of the minute palm side up.
5. After the 1st min, the panelist repeated step 4 without placing hands in the bucket of water.
6. After the 2nd min, the panelist dropped the sponge in the bucket of water and squeezed the sponge in the water to generate more lather as well as to immerse their hand in the solution. Then the hand with the sponge was taken out of the solution and the sponge was squeezed for 5 s to generate enough lather to cover the hand. After the panelist had generated enough lather, the sponge was dropped back into solution and the hand remained out of solution for the remainder of the 3rd min.
7. Step 6 was repeated twice.

Table 1. Grading scale for overall hand condition

Grading scale	Feature
10	Essentially perfect skin
9	Fairly generalized powdery scales primarily on the dorsum
8	Patchy to generalized small scales on the dorsum, and/or in the interdigital and volar digital crease area
7	Generalized large lifted scales on the dorsum, interdigital web and palmar digital areas
6	Localized to generalized large lifted scales over the entire dorsum, possibly with some shallow fissures

Table 2. Dry hand-skin grading scale (10)

Grading scale	Feature
0	No dry skin.
1	Slightly lifted or powdery scales in only one area: on dorsum, palmar side or between the fingers.
2	Slightly lifted scales, covering 50–100% of the surface of two or more areas (dorsum, palmar or between fingers).
3	Moderately lifted or powdery scales in only one area: on dorsum, palmar side or between fingers.
4	Moderately lifted scales, covering 50–100% of the surface of two or more areas (dorsum, palmar or between fingers).
5	Highly lifted or powdery scales in only one area: on dorsum, palmar side or between the fingers.

8. After 5 min, the panelist rinsed their hands in the same specific water conditions until the panelist felt solution and product were rinsed off completely, and the hands were patted dry.
9. Steps 2–8 were repeated two more times for a total of 15 min. This procedure was repeated on 4 (Japan) or 9 (Korea) consecutive days.

Measurement

Both hands of the panelists were evaluated 1 h after Step 9 was completed. The experienced clinical examiner was blinded to study group conditions. Hands were graded for overall condition and degree of skin dryness based on the grading scale shown in Tables 1 and 2. The panelists had stayed in the room for at least 10 min before the clinical measurement starting. The overall hand condition and hand skin dryness of all panelists were graded prior to the hand exposure on Day 1 to get the initial grade value.

Data analysis

At the conclusion of the test, the overall hand-skin condition, hand-skin dryness and capacitance data were presented as means $\pm$ SE. Statistical significance was evaluated with ANOVA.

Results

Results of DAHIT with Korean panelists:

Figure 1 shows the time- and dose-dependent effects on overall hand-skin conditions after exposure to the three test solutions. There were statistically significant differences ($p < 0.05$) in overall

hand grades between 0.02% protease and control (days 6, 7 and 8) and between 0.005% protease and control (day 7). All average scores from days 4–9 of enzyme groups (0.005% and 0.02%) were directionally higher (better) than the scores of non-enzyme control. Though there was no statistical difference, the average scores of 0.02% protease group were higher than those of 0.005% protease group, showing the dose-response trend of protease on the skin conditions throughout the study. Overall, 0.02% protease-containing product caused the least adverse effect to the hand skin among the three products. The non-protease control resulted in the worst overall skin hand condition.

Figure 2 shows the time- and dose-dependent ef-

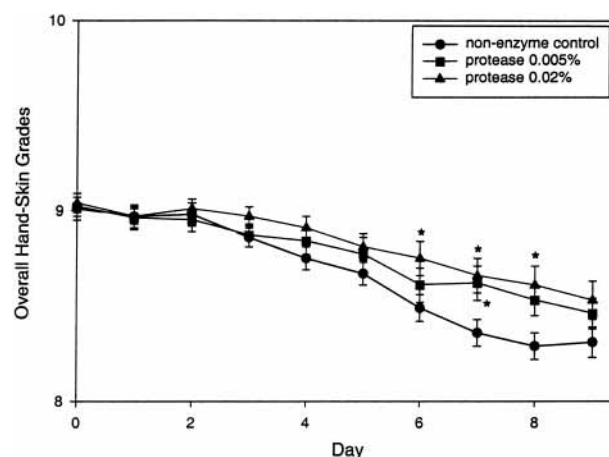


Fig. 1. Time- and dose-dependent effects of protease-containing dishwashing solutions on overall hand-skin conditions in Korean panelists. *Statistically significant difference ($p < 0.05$) between the non-enzyme control and protease-containing solutions.

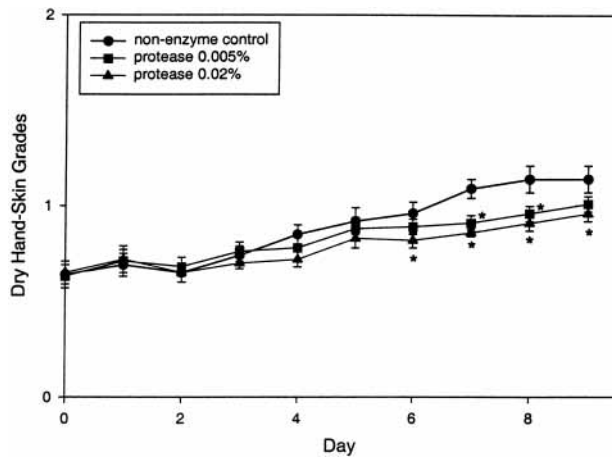


Fig. 2. Time- and dose-dependent effects of protease-containing dishwashing solutions on dryness of hands in Korean panelists. *Statistically significant difference ($p < 0.05$) between the non-enzyme control and protease-containing solutions.

fects on dry hand-skin conditions after exposure to the three test solutions. There were statistically significant differences ($p < 0.05$) in dry hand skin grades between 0.02% and control (days 6–9) and between 0.005% and control (days 7 and 8). The average scores from days 4–9 of protease groups (0.005% and 0.02%) are directionally lower (less dry) than the scores of non-protease control. This means that the non-protease control resulted in more skin dryness than the protease group. Though there was no statistical difference, the 0.02% protease group was directionally better in keeping the skin less dry than the 0.005% protease group, showing the dose-response trend of protease during the study.

Results of DAHIT with Japanese panelists

Figure 3 shows the time- and dose-dependent effects of protease-containing DWL products on overall hand-skin conditions in Japanese panelists including both normal and atopic subjects. There were statistically significant differences ($p < 0.05$) in overall hand grades between the protease group and control (days 3 and 4). All average scores of the protease group were directionally higher (better) than the scores of non-enzyme control. Protease-containing products caused less adverse effect to overall hand-skin conditions than non-enzyme products.

Figure 4 shows the time- and dose-dependent effects of protease-containing DWL products on dry hand-skin conditions in Japanese panelists including both normal and atopic subjects. There were statistically significant differences ($p < 0.05$) in dry hand skin grades between protease and control (days 2 and 4). The average scores of the 0.005%

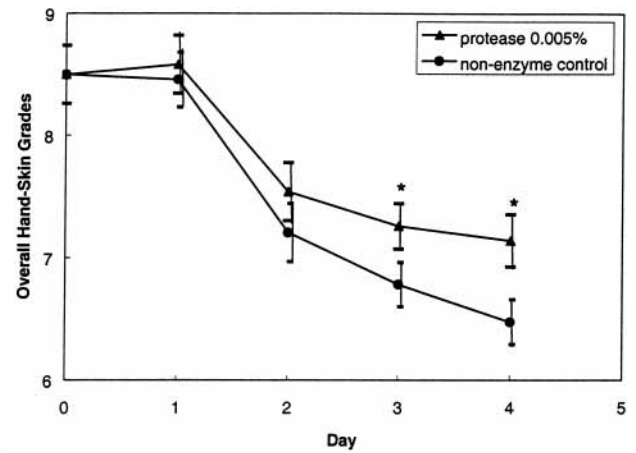


Fig. 3. Time- and dose-dependent effects of protease-containing dishwashing solutions on overall hand-skin conditions in Japanese panelists (both normal and atopic). *Statistically significant difference ($p < 0.05$) between the non-enzyme control and protease-containing solutions.

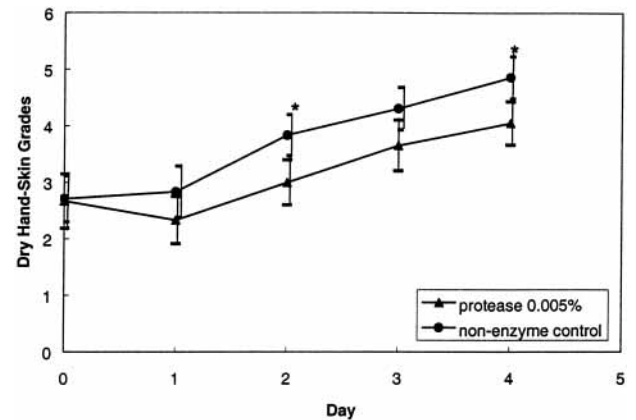


Fig. 4. Time- and dose-dependent effects of protease-containing dishwashing solutions on dryness of hands in Japanese panelists (both normal and atopic). *Statistically significant difference ($p < 0.05$) between the non-enzyme control and protease-containing solutions.

protease group were directionally lower (less dry) than the scores of non-protease control during the study. These data indicate that the 0.005% protease group had directionally better skin (less dry) than non-enzyme control group.

Figure 5 shows the time- and dose-dependent effects of protease-containing dishwashing solutions on overall hand-skin conditions of atopic Japanese panelists. There was a statistically significant difference ($p < 0.05$) in overall hand grades between the protease group and control (day 4). All average scores of the protease group were directionally higher (better) than the scores of non-enzyme control throughout the study.

Figure 6 shows the time- and dose-dependent effects of protease-containing dishwashing solutions

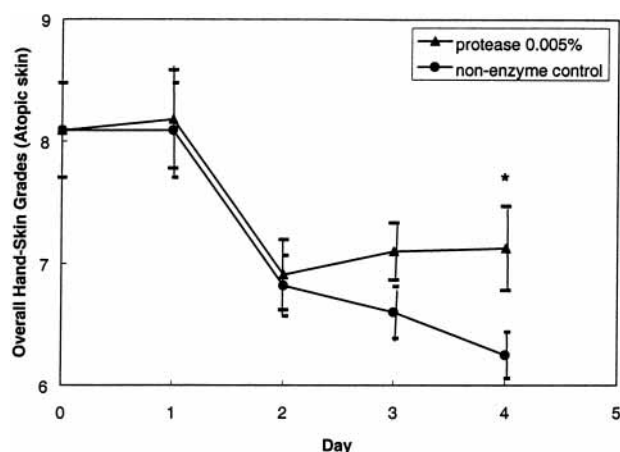


Fig. 5. Time- and dose-dependent effects of protease-containing dishwashing solutions on overall hand-skin conditions in atopic Japanese panelists. *Statistically significant difference ($p < 0.05$) between the non-enzyme control and protease-containing solutions.

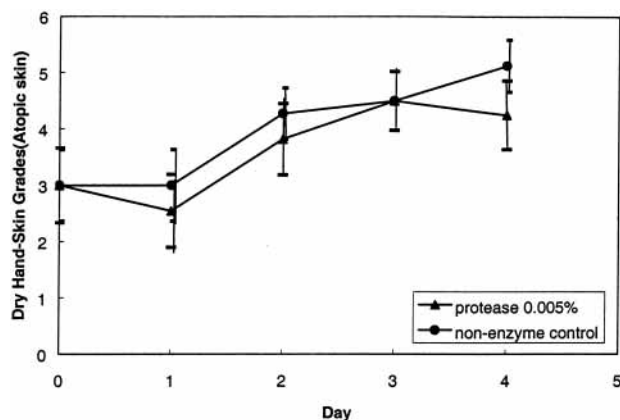


Fig. 6. Time- and dose-dependent effects of protease-containing dishwashing solutions on dryness of hands in atopic Japanese panelists. *Statistically significant difference ($p < 0.05$) between the non-enzyme control and protease-containing solutions.

on dryness of hands in atopic Japanese panelists. There were no statistically significant differences ($p < 0.05$) in dry hand skin grades between protease and control during the study. Although there were no statistical differences, the average scores of the 0.005% protease group are directionally lower (less dry) than the scores of non-protease control throughout the study. The data indicate that the 0.005% protease group did not keep the skin less dry than the non-enzyme control group.

Discussion

The objective of the studies with Korean and Japanese panelists was to show no adverse effects on the skin from using protease-containing DWL compared to the non-enzyme containing control.

The results of both Korean and Japanese studies show that DWLs containing protease (0.005% and 0.02%) do not cause adverse skin effects to the subjects compared with non-enzyme control during the studies. Furthermore, the study results show that the protease-containing DWLs are better than non-protease control in maintaining the original overall and dry hand-skin conditions. These findings are consistent with the report from Loomans & Hannan (11), which showed that the intact cells of the stratum corneum are resistant to proteolytic attack by subtilisin and that this resistance is not affected by the presence of surfactant at the concentrations used in these studies.

Proteins and lipids are key building blocks of the epidermis, and therefore may act as substrates for protease and lipase in detergents. At higher concentrations of active proteases in detergents, the protective functions of the stratum corneum and epidermis can be damaged by routine cleaning applications. However, lower concentrations of protease in DWL can better improve skin conditions by effectively removing superannuated stratum corneum from the skin without damaging intact skin cells. Therefore, it can be inferred that the skin irritation capacity of enzyme-containing detergents is highly dependent on the concentration of enzymes used in detergent products.

In the direct application hand immersion studies, 0.005% and 0.02% protease in DWL did not cause any adverse effects on skin conditions. The data show that the DWLs containing protease resulted in hand-skin conditions that were better than the hand-skin conditions of those panelists using the non-enzyme control product. In addition, 0.005% protease in the detergent liquid matrix was not associated with irritation issues to the atopic skin of Japanese panelists, and actually resulted in skin conditions that were better than the group using the non-enzyme DWL. The results of no skin irritation in the atopic subjects who were exposed to the low levels of protease-containing DWL is consistent with the report from Andersen et al. (8) which showed no significant irritant capacity of detergent enzyme consisting of protease at 1%, lipase at 0.4%, and amylase at 0.4% in atopic dermatitis patients.

It seems that seasonal temperature variation affected skin responses to the products. The low temperature (5–10°C) in early March in Japan caused overall slightly worse skin conditions and skin dryness than the higher temperature (15–23°C) in late April/early May in Korea. However, the temperature difference did not change the overall effects of protease versus non-enzyme products on the skin.

Based on the study results, a low level of pro-

tease use in DWL does not cause adverse effects on either normal or atopic skin. The data in this paper show that the low level of protease is beneficial for maintaining hand-skin condition that is better than the hand-skin condition of those using a non-enzyme containing DWL.

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