Modifying Different Types of Flours to Deter the Growth of Bacteria

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***Abstract*— The purpose of this research was to discover a certain starch or protein solution from Gluten-Free, genetically modified, and normal flour that could effectively inhibit bacterial growth without harming humans. The discovered solution can be used instead of chemicals to wash vegetables and fruits. The effects of the solutions were first tested directly on soil bacteria and then on *E. coli.* Then, the effects of the solutions were tested on plants and fungi. It was proven that bacteria on contaminated organic plants are not easily washed away by injecting *E. coli* on raw vegetables and it was tried in this experiment to show the effects of the solutions by injecting them on the contaminated vegetables and measuring the number of remaining bacteria. The results of the experiments showed that solutions 4 and 12 were effective at inhibiting the growth of bacteria while being safe for humans.**

Keywords—flour, solution, E. coli, inhibit

A. INTRODUCTION

Recently, a young girl who ate a undercooked burger from McDonalds got infected with Escherichia Coli (*E. coli*) O157 and suffered from a kidney failure caused by hemolytic-uremic syndrome (HUS).

Similarly, 151 people in the United Kingdom had been infected with the same strain of *E. coli* from eating salad leaves contaminated with the same strain of bacteria.

To prevent themselves getting harmed by bacteria, people have been using a lot of chemicals. The chemicals were remarkable to people because they acted as an antibiotic to vegetables, making the vegetables safer for people to eat raw. However, not only do these chemicals accumulate in our body, but they also contaminate the soil or run off and create algal bloom, which both harm the environment.

B. GOAL

The aim of this project is to figure out a specific solution modified from three different types of flours – Gluten-Free, genetically modified, and normal – that can inhibit the growth of bacteria while staying completely harmless to humans. The effective solution discovered can be used to wash vegetables and fruits safely than chemicals.

C. PROCEDURE

A. Extracting protein and starch molecules from three types of flour

1) Prepare Gluten-Free, GMO, and normal flour. Measure 10 grams of each flour mix it with 100mL of water in an Erlenmeyer flask.
2) Transfer 50mL of each solution into conical tubes.
3) Put the conical tubes in a centrifuge under 10,000 rotations per minute for 30 minutes.
4) Filter the solutions with 0.2 ㎛ pores to isolate macromolecules. There will be 12 solutions.

B. Changing the structure of three types of flour solutions

1) Heat 250mL of distilled water up to 60-70℃.
2) Warm half of the modified flour solutions, 6 tubes, in the warm water.
3) In 12 micro tubes, put 1mL of each modified flour solution. Then, take half, 6 tubes, and inject 200 ㎕ amylase or protease to modify them further.

C. The effects of modified flour solutions on the growth of soil bacteria

1) Inject 5 ㎕ of NB medium where aerobic or anaerobic bacteria were cultured to 1mL of NB medium in a tube. Then, inject 200 ㎕ of modified flour solutions in the same tube.
2) Incubate the tube mentioned above in 28℃ for 12 hours.
3) Measure the growth of aerobic and anaerobic bacteria with UV-Spectrophotometer under the wavelength of 630nm.

D. The effects of flour solutions on the growth of E. coli

1) Inject 5 ㎕ of NB medium that contains *E. coli* to 1mL of NB medium. Then, inject 200 ㎕ of modified flour solutions.
2) Incubate *E. coli* in the modified flour solutions in 37℃ for 12 hours.
3) Put *E. coli* in the UV-Spectrophotometer under the wavelength of 630nm to measure their growth.

E. The effects of modified solution on the growth of fungi

1) Soak chromatography papers in the modified flour solutions and place them on the PDA medium.
2) Put fungi spores on the same PDA medium.
3) Let the fungi grow for a week in a dark place and observe their growth.

F. The effects of modified solutions on the growth of plants

1) Mix 0.7 grams of agarose gel powder with 100mL of distilled water to apply on the plants' roots.

2) Plant 12 cabbage seeds on each gel and let them grow for a week.
3) Cut the leaves and the stems of the cabbages and put them in the modified flour solutions.
4) After incubating them in 4℃ for 2 hours, put them in UV-Spectrophotometer under the wavelength of 633 and 645nm to measure the changes in the amounts of chlorophyll a and b.

G. The effects of modified flour solutions on the growth of E. coli on plants

1) Plant 12 cabbage seeds on sterilized soils and inject *E. coli* and the 12 flour solutions on the soil.
2) After a week, cut the plants' leaves and the stems and put them in NB medium to incubate in 37℃ for 12 hours.
3) After incubation, put the cabbages in UV-Spectrophotometer under the wavelength of 630nm to measure the growth of *E. coli.*

H. The contamination of E. coli on plants before and after washing the plants

1) Prepare radish sprouts, sprouts, lettuce, and perilla leaves and inject 5 $\mu\ell$ of NB medium with *E. coli.*
2) Incubate them in 4, 24, and 37℃ for 12 hours.
3) Take the vegetables out and wash them thrice, in one experiment with distilled water and in the other with the 12 flour solutions.
4) Put the washed vegetables in the NB medium and incubate the washed vegetables once more and take them out after 12 hours
5) Put the incubated vegetables in NB medium in the UV-Spectrophotometer in order to measure the growth of *E. coli* under the wavelength of 630nm.

D. Result

A. Extracting protein and starch molecules from three types of flour

Modified flour solutions from Gluten-Free, GMO, and normal flour are refrigerated.

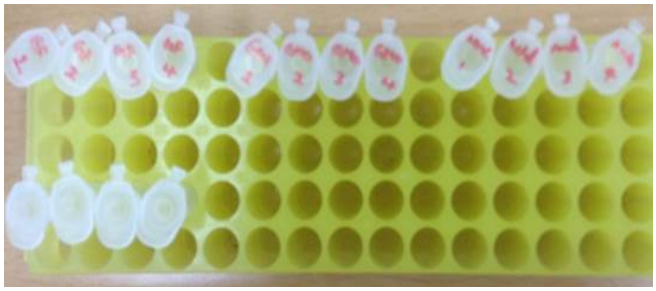


Fig. 1. 1.5mL micro-tubes containing protein and starch solutions from Gluten-Free, GMO, and normal flour

B. Changing the structure of three types of flour solutions

Amylase changes the structure of starch, and protease changes the structure of protein. A temperature between 60 and 70℃ modifies the structure of Gluten-Free, GMO, and normal flour.

	amylase	protease	amylase + heat	protease + heat
Gluten-Free wheat flour	1	2	3	4
wheat flour	5	6	7	8
GMO wheat flour	9	10	11	12

Fig. 2. Numbers assigned to 12 modified flour solutions

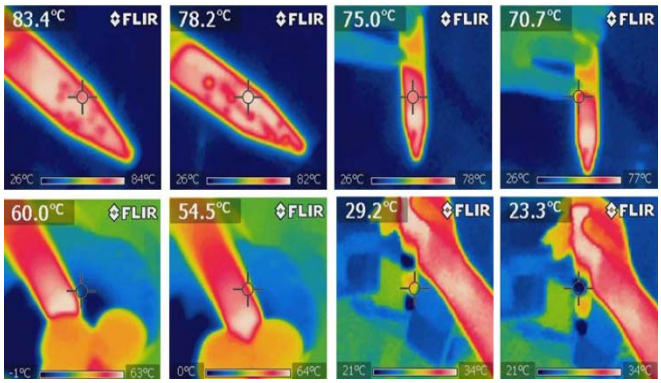
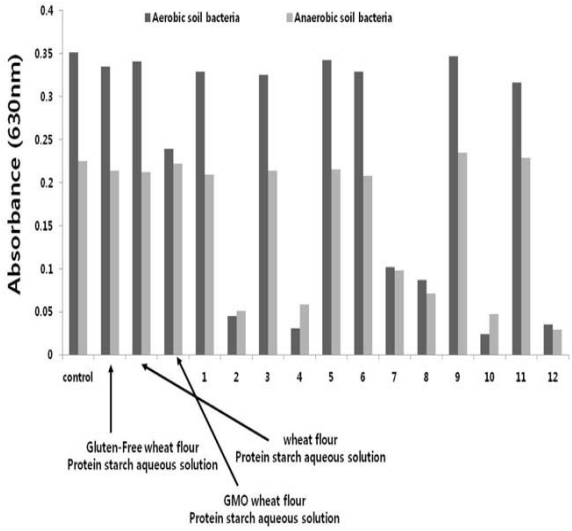


Fig. 3. Thermal Infrared cameras indicate temperatures of the solutions

C. The effects of modified flour solutions on the growth of soil bacteria



	amylase	protease	amylase + heat	protease + heat
Gluten-Free wheat flour	1	2	3	4
wheat flour	5	6	7	8
GMO wheat flour	9	10	11	12

Fig. 4. The effects of flour solutions on the growth of soil bacteria

Solutions 2,4,7,8,10, and 12 have the ability to inhibit the growth of the soil bacteria. The NB medium that contain solutions listed above have the ability to restrict the growth of both aerobic and anaerobic soil bacteria.

D. The effects of flour solutions on the growth of E. coli

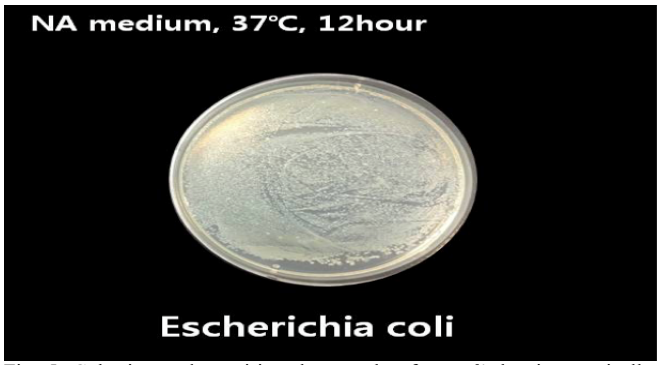


Fig. 5. Culturing and acquiring the sample of *E. coli* that is genetically modified so that they stay safe to humans

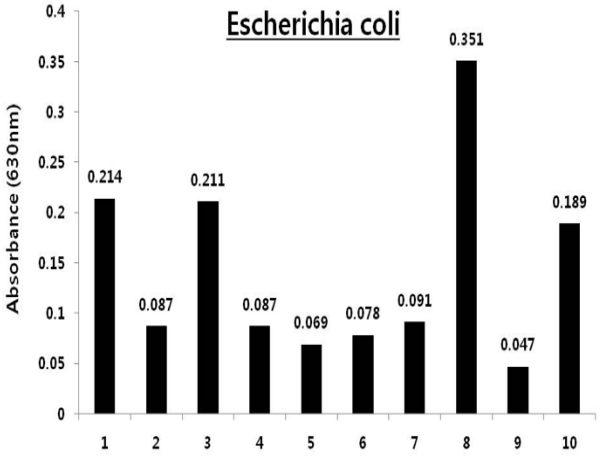
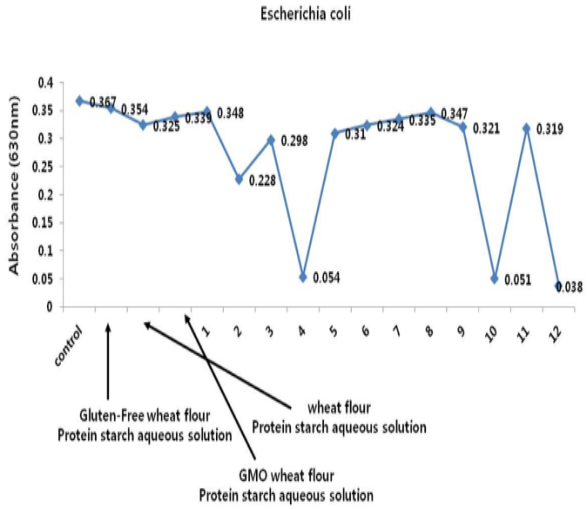


Fig. 6. Measuring the growth of *E. coli* with UV-Spectrophotometer



	amylase	protease	amylase + heat	protease + heat
Gluten-Free wheat flour	1	2	3	4
wheat flour	5	6	7	8
GMO wheat flour	9	10	11	12

Fig. 7. The effects of modified flour solutions on the growth of *E. coli*

Solutions 4, 10 and 12 have the ability to inhibit the growth of *E. coli*. This was verified because NB medium combined with these solutions listed above had the ability to prevent the growth. Solution 2, which inhibited the growth of soil bacteria, could not inhibit the growth of *E. coli.*

E. The effects of modified solutions on the growth of fungi

	amylase	protease	amylase + heat	protease + heat
Gluten-Free wheat flour	1	2	3	4
wheat flour	5	6	7	8
GMO wheat flour	9	10	11	12

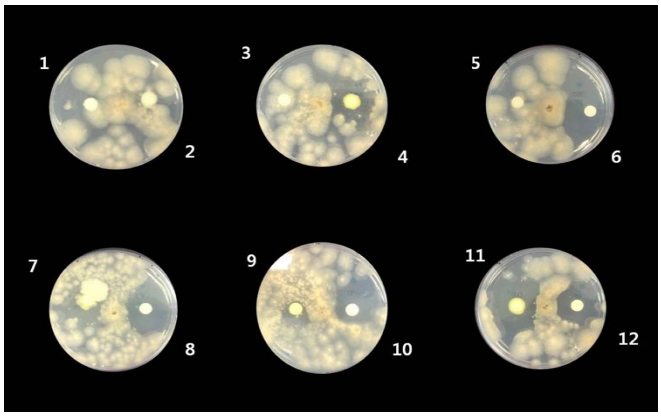


Fig. 8. The effects of modified flour solutions on the growth of fungi

It is more important to observe the effects of modified flour solutions on the growth of fungi, a eukaryotic organism, because fungi are more related to humans than prokaryotic bacteria. For modified solutions from Gluten-Free, GMO, and normal flour, which already inhibited the growth of prokaryotic bacteria and potentially be used as an antibiotic, it is necessary to test out the solutions' effects to eukaryotic organisms because if they are harmless to fungi and plants, it is more likely that they will stay harmless to humans. For example, a pill for tinea pedis, a condition caused by fungal infection, is much more effective than ointments in curing the condition, but doctors rarely prescribe the pill to people because pills that harm fungi also harm human liver. According to the experiment, solutions 4,6,8,10,11, and 12 had the ability to prevent the growth of fungi. However, this result also opens up the possibility that the solutions listed above may harm humans, and therefore, further experiments are required on plants in order to verify the safety of the flour solutions on humans.

F. The effects of modified solution on the growth of plants

From the fungi experiment, it was implied that the solutions that inhibit the growth of fungi could also harm eukaryotic organisms like humans. Testing the effects of the solutions to plants, another organism closer to humans than prokaryotic bacteria, was the best way to test the effects of the solutions one more time. It was learned that the solutions, which harmed the fungi, did not harm the plants' growth through measuring the amount of chlorophyll a and

b in each plant with UV-Spectrophotometer. If the solution was harmful for plants, it would have inhibited the growth of plants and reduced or changed their chlorophyll a and b levels.

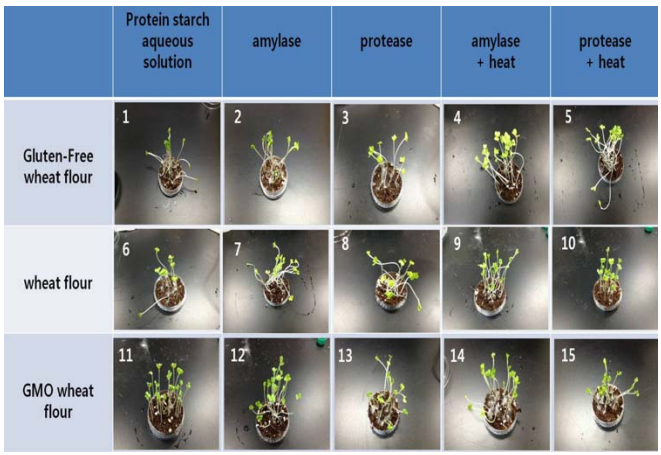


Fig. 9. The effects of modified flour solutions on the growth of cabbage

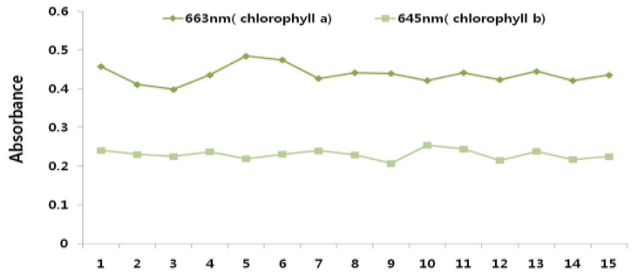


Fig. 10. The amount of chlorophyll a and b in plants measured through UV-Spectrophotometer in order to find out the effects of modified flour solutions on the growth of cabbage

From the fungi experiment, it was implied that the solutions that inhibit the growth of fungi could also harm eukaryotic organisms like humans. Testing the effects of the solutions to plants, another organism closer to humans than prokaryotic bacteria, was the best way to test the effects of the solutions one more time. It was learned that the solutions, which harmed the fungi, did not harm the plants' growth through measuring the amount of chlorophyll a and b in each plant with UV-Spectrophotometer. If the solution was harmful for plants, it would have inhibited the growth of plants and reduced or changed their chlorophyll a and b levels.

G. *The effects of modified flour solutions on the growth of E. coli on plants*

	amylase	protease	amylase + heat	protease + heat
Gluten-Free wheat flour	1	2	3	4
wheat flour	5	6	7	8
GMO wheat flour	9	10	11	12

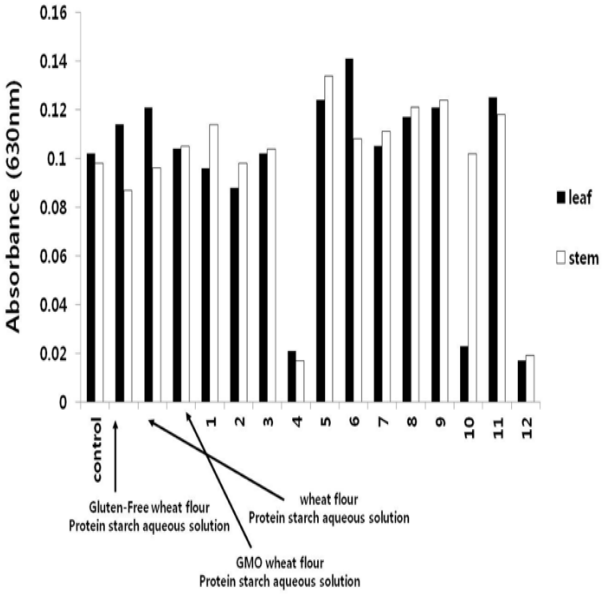


Fig. 11. The effect injecting modified flour solutions on the sterilized soil has on the contamination of plants that grew on the *E. coli* contaminated soil.

It is not well known to other people that vegetables that grew on *E. coli* contaminated soil have a high chance of getting contaminated with *E. coli*. The purpose of this experiment was to find out the ability of the modified flour solutions in preventing the contamination of *E. coli* from the soil that the plants grew on. From the experiment, it was learned that solutions 4 and 12, when injected to the sterilized soil, had the ability to prevent the contamination of *E. coli* on plant's leaves and stems.

H. *The contamination of E. coli on plants before and after washing the plants*

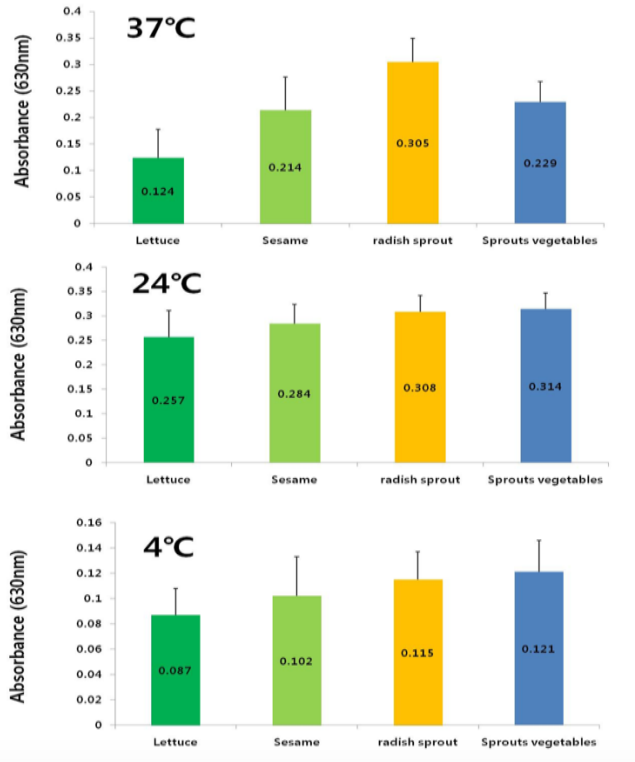


Fig. 12. The growth of *E. coli* after washing the contaminated plants

4 different plants contaminated with *E. coli* which were stored in temperatures 4, 24, and 37℃ have been washed 2 times, and the growth of the remaining *E. coli* has been closely observed. The results suggested that *E. coli* were never fully washed from all 4 plants, and the remaining bacteria multiplied. Therefore, it was confirmed that through normal process of washing vegetables, it is impossible to wash away *E. coli* completely if the vegetables were contaminated with *E. coli.*

	amylase	protease	amylase + heat	protease + heat
Gluten-Free wheat flour	1	2	3	4
wheat flour	5	6	7	8
GMO wheat flour	9	10	11	12

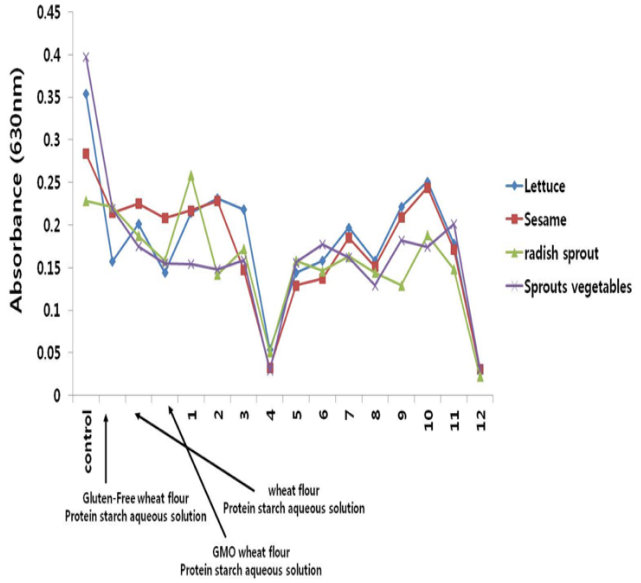


Fig. 13. The effect injecting protein and starch solutions on the contaminated vegetables have on the growth of *E. coli*

From the experiment, it was confirmed that it is very hard to wash away *E. coli* from 4 types of vegetables that were contaminated. However, *E. coli* contamination did not happen to the vegetables that were injected with solutions numbers 4 and 12 before they were washed because the E. coli was successfully removed while washed.

CONCLUSION

The world is growing with hunger to find cures for bacterial diseases. From recent controversies about bacteria unwashed on raw foods to harms of chemicals, conducting a research on a natural and effective antibacterial solution that is healthier than the current means of fighting off bacterial diseases seemed imperative. A solution extracted from natural ingredients that can limit the growth of bacteria can be substituted into people's lives instead of chemicals. The aim of this experiment was to extract protein and starch solutions from Gluten-Free, GMO, and normal flour, modify them with heat and enzymes, and inject them to different types of bacteria to test the capability of the solution to inhibit the growth of bacteria. From the experiment, a few effective protein or starch solutions in dealing with bacteria in various places and organisms were discovered. To find out if the protein and starch solutions from Gluten-Free, GMO, and normal flour can successfully deal with prokaryotic bacteria while being harmless to eukaryotic organisms, experiments were done to fungi and plants. The solutions discovered harmed the fungi but succeeded in preventing the growth of bacteria on plants and vegetables. It is still safe to use the solutions because even though the solutions harmed fungi, they had no effect on the plant growth. In addition, the interesting result lead into a different prospect – the solutions can be used to deal with fungal diseases such as athlete's foot because they inhibited the growth of fungus. There may be numerous ways the solutions inhibited bacteria, but only one possibility makes the most sense – the solutions are successful at blocking bacteria's transcription process. The components of the solution interrupted the process of *E. coli*'s transcription and translation, modifying the DNA structure of *E. coli* and repeating the process permanently affecting *E. coli*. Next time, an investigation on how bacteria were inhibited should be done by doing DNA tests and focusing on the genetic area. Also, it is necessary to discover a better way to preserve the twelve solutions because in this experiment, the solutions had to be remade often. Lastly, there is a need to test the effects of protein and starch solutions on human skin or on animals because that way, more accurate results are obtainable.

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