Title: PREPARATION METHOD OF FERMENTED PRODUCTS OF FRUITS OR VEGETABLES, FERMENTED PRODUCTS PREPARED THEREBY AND FUNCTIONAL COMPOSITION COMPRISING THE SAME

Abstract: The present invention relates to a preparation method of fermented products of fruits or vegetables, fermented products prepared thereby and functional composition comprising the same. More particularly, the present invention relates to a preparation method of fermented products of fruits or vegetables comprising the step of injecting and fermenting the fruits, vegetables or the mixtures thereof with yeast and lactobacillus in stages, fermented products prepared thereby and functional composition comprising the same. The fermented products prepared by the method according to the present invention contain plenty of lactobacillus with good acid-resistance and bile tolerance, and physiologically active components such as organic acids, enzymes or vitamins which are useful in the human body, then being excellent in the antimicrobial activity, the probiotic activity and the deodorant activity as compared with the fermented products injected with yeast or lactobacillus alone. Therefore, the fermented products according to the present invention are very useful as a ingredient of functional food, diet food, antimicrobial, probiotic and feed composition.
Description

PREPARATION METHOD OF FERMENTED PRODUCTS OF FRUITS OR VEGETABLES, FERMENTED PRODUCTS PREPARED THEREBY AND FUNCTIONAL COMPOSITION COMPRISING THE SAME

Technical Field

[1] The present invention relates to a preparation method of fermented products of fruits or vegetables, fermented products prepared thereby and functional composition comprising the same. More particularly, the present invention relates to a preparation method of fermented products of fruits or vegetables comprising the step of injecting and fermenting the fruits, vegetables or the mixtures thereof with yeast and lactobacillus in stages, fermented products prepared thereby and functional composition comprising the same.

[2] Background Art

[3] Fruits or vegetables have lots of nutrients such as vitamins, minerals and cellulose, and each has its own taste and aroma. In particular, since the fruits or vegetables contain a great quantity of cellulose, they facilitate probiotic activities and evacuation and are effective in diets by making feel satisfied. Moreover, fruits or vegetables are considered as foods for health and beauty, because skin and seeds of fruits or vegetables have various polyphenols which have good anti-oxidation effects.

[4] However, the skin and seeds of fruits or vegetables have unpleasant taste and limits as a food in itself. In addition, fruits or vegetables have disadvantages, i.e. those have seasonal limits and short shelf life, and their various effective components are changed or lost their function by the transpiration, respiration and after-ripening during the storage period. To solve these problems, various food processing methods for fruits or vegetables have been developed in many ways.

[5] In particular, the preparation methods of processed food which has enhanced physiological activities by injecting and fermenting fruits or vegetables with lactobacillus have been recently developed. For Example, Korean Patent No. 319377 discloses fermented beverage prepared by fermenting the extract of vegetables and oriental medicinal stuff with Lactobacillus plantarum, and Korean Patent No. 167863 discloses preparation method of composite enzyme beverage containing solution prepared by fermenting medicinal herbs, fruits extract and crops with lactobacillus. Also, Korean Patent No. 185250 discloses preparation method of fruits or vegetables beverage
containing lactobacillus, by preparing vegetable solution by fermenting the vegetable extract with *Lactobacillus plantarum*, and then optionally adding fruit juice or vegetable extract into the fermented solution. However, these methods mainly relate to the fermented beverages which are produced by fermenting the extract of fruits or vegetables with lactobacillus.

Disclosure of Invention

Technical Problem

Accordingly, the present inventors have conducted studied to prepare fermented products of fruits or vegetables comprising lactobacillus having excellent acid resistance and bile tolerance. As a result, the present inventors have obtained fermented products of fruits or vegetables prepared by injecting and fermenting the fruits, vegetables or the mixtures thereof with yeast and lactobacillus in stages and has completed the subject invention. The fermented products of fruits or vegetables comprise plenty of lactobacillus having excellent acid resistance and bile tolerance, and physiologically active components such as organic acids, enzymes or vitamins which are useful in the human body, then being excellent in the antimicrobial activity, the probiotic activity and the deodorant activity as compared with the prior fermented products prepared by injecting and fermenting the fruits, vegetables or the mixtures thereof with yeast or lactobacillus alone.

Accordingly, an object of the present invention is to provide a preparation method of fermented products of fruits or vegetables comprising the step of injecting and fermenting the fruits, vegetables or the mixtures thereof with yeast and lactobacillus in stages, fermented products prepared thereby and functional composition comprising the same.

Technical Solution

To accomplish the object, the present invention provides the preparation method of fermented products of fruits or vegetables comprising the step of injecting and fermenting the fruits, vegetables or the mixtures thereof with yeast and lactobacillus in stages.

The present invention further provides the fermented products prepared by said method.

Also, the present invention provides the composition comprising said fermented products.

The present invention will now be explained in detail.

The preparation method of fermented products of fruits or vegetables according to
the present invention is characterized in firstly fermenting fruits, vegetables or the mixtures with yeast and secondly fermenting those with lactobacillus so that the results have a low pH and contain physiologically active components which are useful in the human body such as organic acids and lactobacillus in great quantities as compared to the products injected and fermented with yeast or lactobacillus alone.

Also, the fermented products prepared by said method can be applied in various fields by separating extracted juice and solid and processing the solid by drying and grinding to fine powder. Moreover, in the preparation method according to the present invention, the skin or seeds of fruits or vegetables are used in itself so that it is possible to prepare the fermented products containing all kinds of physiologically active components and nutrition in the skin or the seeds.

More specifically, the preparation method of fermented products according to the present invention is characterized in firstly injecting and fermenting fruits, vegetables or the mixtures with yeast and secondly injecting and fermenting those with lactobacillus. In one experimental example, the changes of physiologically active components contained in the fermented products which were prepared by firstly injecting and fermenting the mixtures of fruits or vegetables with yeast and secondly injecting and fermenting those with lactobacillus have been determined. This results were compared with the changes in the case of being prepared by vice versa, i.e. firstly injecting and fermenting the mixtures with lactobacillus and secondly injecting and fermenting those with yeast(see the experimental Example <1-1>). The result shows that the products of the former case have a low pH and a high lactic acid content and more than ten times of lactobacillus than those of the latter case(see Table 1).

Preferably, the preparation method of the fermented products of fruits or vegetables according to the present invention comprises the steps:

(a) grinding fruits, vegetables or the mixtures thereof, and then mixing them with purified water and sugar;
(b) injecting and fermenting the mixture of step (a) with yeast; and
(c) injecting and fermenting the fermented products of step (b) with lactobacillus.

The preparation method of the fermented products according to the invention will be explained step-by-step hereinafter.

(a) **Grinding raw materials and adding purified water and sugar**

The raw materials of the products according to the present invention can be fruits, vegetables or the mixtures. It is preferable to use fruits or vegetables which have a high polyphenol content in skin or seeds.

Examples of fruits are apples, pears, oranges, bananas, persimmons, grapes, kiwis, pineapples, strawberries and so on.

Apples contain vitamin C, minerals and organic acid etc. more than other fruits.
Also, apples are effective against constipation with help of plenty of cellulose and pectin and are known to have anti-inflammatory effects because of the malic acid which is one of the organic acids contained in apples. Anthocyanin pigments contained in the skin of apples have excellent anti-oxidation effects. Pears contain pentosan and citric acid and has a high protease content. The skin of oranges has a high carotene content and the flesh of oranges have excellent anti-oxidation effects with help of a high vitamin C and hesperidin content. Bananas have a high water-soluble sugar content and contain organic acids such as citric acid and malic acid. Particularly, the flesh of well ripe bananas have anti-oxidation effects with a high carotin content, and are effective for the evacuation with help of a high pectin and cellulose content. Sweet persimmons have plenty of beta-carotin which is excellent in anti-oxidation, and have a high vitamine C content. Grapes also have excellent anti-oxidation effects since they contain anthocyanin pigments and their seeds contain polyphenol.

Examples of vegetables are tomatoes, cabbage, kale, carrots, broccoli, green onions, onions and so on. Green onions and onions can be added in a small quantity. Such vegetables are also health foods containing a high nutrition and physiologically active components content.

It is preferable to use those fruits or vegetables only after washing without removing the skin or the seeds. It is also preferable to wash with the detergent for fruits or vegetables firstly and then with diluted vinegar secondly.

The washed fruits or vegetable are grinded and then mixed. It is preferable that fruits or vegetables are mixed in the same quantity.

Purified water is added to the grinded materials. It is intended to blend the grinded materials and to dilute polyphenol and weaken the antimicrobial activity of polyphenol after the injection of yeast or lactobacillus. Preferably, purified water is added at 60-65% by weight.

Also, sugar is added to the grinded materials to regulate the sugar content. This is intended to provide nutrition for microorganism and to accelerate the fermentation.

Any sugar can be used if it is usable as a food additive. The sugar can be dextrose, fructose powder, liquefied high fructose, sugar, maltose, lactose and oligosaccharide etc separately or in combination. Brown sugar is preferable.

It is preferable that the sugar content is between 10 and 14 brix. If the sugar content is less than 10 brix, it can not accelerate the growth of the microorganism, while if the sugar content is more than 14 brix, the microorganism is restrained of the growth with the effect of the osmotic pressure and the fermentation rate is decreased.

After the addition of sugar, agitation is performed by the traditional agitator to blend the grinded materials of fruits or vegetables.

(b) The first fermentation using yeast
Fruits, vegetables or the mixtures regulated with sugar content in the step (a) are injected and fermented with yeast firstly. The fermentation using yeast is intended to produce the organic acid, to establish the sour taste and to provide the final fermented products with the yeast as probiotics. Also, it is intended that lactobacillus in the second fermentation use organic acid and sugar which were produced in the first fermentation by yeast, thereby increasing the content of useful materials and organic acid in the final fermented products.

The "probiotics" are living microbes, that is to say, microorganisms which can live in the stomach and intestines and can prevent or treat the specific pathological conditions in human and animals. More specifically, it is a medicine or an animal medicine which are prepared with live microorganisms. Generally, probiotics can treat and improve the various conditions which are caused by the abnormal fermentation of bacterial flora in the intestines, can also be accumulated and fixed in the wall of the digestive canal of the intestines to prevent the harmful bacteria from fixing in the digestive canal of the intestines, and can produce the lactic acid to lower the pH in the inside of the intestines and to prevent the harmful bacteria from proliferating. Moverover, the probiotics produce the peroxyde with Bacteriocin, so inhibit the growth of the germs and help the activity of villi absorbing the nutrition. Besides, the probiotics produce the materials which help the absorption and the use of nutrition, improve the necessary rate of feed in case of animals, and produce the materials which neutralize the toxic materials produced by the germs.

It is preferable to use the yeast which is excellent in the productivity of organic acid, sour taste, vitamins or nucleic acid materials. Preferably, Saccharomyces cerevisiae, Saccharomycopsisfibuligera or the mixture thereof can be used as yeast. More preferably, said Saccharomyces cerevisiae can be Saccharomyces cerevisiae KCCM10741P, and said Saccharomycopsisfibuligera can be Saccharomycopsis fibuligera KCCM 10591.

Preferably, the yeast is injected at 0.5 to 1.2% by weight of the total amount of ingredients. It is preferable that the fermentation is performed at 24-26°C for 6-10 days. More preferably, the fermentation is performed at 25°C for 8 days.

If the fermentation is performed at less than 24°C, there is a disadvantage that the fermentation takes place slowly, while if the fermentation is performed at more than 26°C, there is a disadvantage that the yeast can not be activated and become extinct. In addition, if the fermentation time is less than 6 days, the organic acids and useful materials can not be produced sufficiently, while if the fermentation time is more than 10 days, the amount of the organic acids and useful materials decreases due to the metabolism of themselves.

In an experiment, various fermentations were conducted with different duration time
to determine the optimal fermentation conditions of fruits or vegetables (see Experimental Example <l-2>), the results showed that the number of yeast was the highest and the pH was the lowest in case of the fermentation for 8 days, so the optimal fermentation period is 8 days (see Table 2).

**c) The second fermentation using lactobacillus**

The products fermented with yeast firstly in the step (b) are fermented with lactobacillus secondly. The purpose of the second fermentation with lactobacillus is to provide the products with not only organic acid but also various useful materials such as bacteriocin which are various antimicrobial materials produced by lactobacillus. Moreover, another purpose of the second fermentation is to provide the final products with the lactobacillus as probiotics. Preferably, the lactobacillus can be *Lactobacillus reutri, Lactobacillus acidophilus* and *Lactobacillus brevis* separately or in combination. More preferably, the *Lactobacillus reutri* KCCM 1065 IP, the *Lactobacillus acidophilus* can be *Lactobacillus acidophilus* KCCM 10671 IP and the *Lactobacillus brevis* can be *Lactobacillus brevis* KCCM 10714P.

Preferably, the lactobacillus is injected at 0.5 to 1.2% by weight of the total amount of ingredients. It is preferable that the fermentation is performed at 33-37°C for 10-14 days. More preferably, the fermentation is performed at 35°C for 12 days.

If the fermentation is performed at less than 33°C, there is a disadvantage that the fermentation takes place slowly, while if the fermentation is performed at more than 37°C, there is a disadvantage that lactobacillus cannot be activated and become extinct. In addition, if the period of fermentation is less than 10 days, the strong acid-resistant lactobacillus is not yet activated, while if the period of fermentation is more than 14 days, lactobacillus become extinct and so the number of lactobacillus decreases.

In an experiment, various fermentations were conducted with different duration time to determine the optimal fermentation conditions of fruits or vegetables (see Experimental Example <l-2>), the results showed that the number of lactobacillus was the highest and the pH was the lowest in case of the fermentation for 12 days, so it was identified that the optimal fermentation period is 12 days (see Table 3).

Furthermore, the preparation method of the fermented products according to the present invention can comprises further step of dividing extracted juice and solid from the fermented products.

The extracted juice can be formed to extracts by concentrating or to powder by spray-drying.

The separated solid can be formed to paste, or to fine powder by freeze-drying or hot-air drying and then grinding. It is preferable that the solid is grinded in the particle
size 80 to 120 mesh.

[49] The fermented products prepared by said procedure according to the present invention has the characteristics of a low pH and a high content of physiological active components as compared with the products fermented with only yeast or lactobacillus. In particular, the fermented products prepared by said procedure have been identified to have a higher content of organic acid and lactobacillus than those of the products fermented with only yeast or lactobacillus. Therefore, it can be assured that the combined injection of yeast and lactobacillus can raise the productivity of physiologically active components in the procedure of fermenting fruits or vegetables (see Experimental Example 2 and Table 4).

[50] Moreover, the fermented products according to the present invention have the characteristics of an excellent acid resistance and bile tolerance. One experiment for investigating the acid-resistance and bile acid-resistance of lactobacillus contained in the fermented products according to the invention (see Experimental Example 3) showed a high survival rate of the lactobacillus in 1% of oxgall and pH 2.5 and 3.0 (see Table 5 and 6).

[51] In this manner, the fermented products according to the invention have an excellent acid-resistance and bile tolerance, so that the lactobacillus can be alive in low pH within the body and arrive to the intestines and do the various bio-activities.

[52] The various bio-activities of the fermented products according to the invention have been investigated. In the investigation of antimicrobial activity (see Experimental Example 4), the fermented products according to the invention showed antimicrobial activities against Escherichia coli, Actinobacillus pleuropneumoniae, Salmonella and so on which are the similar level of antimicrobial activity of the known antibiotics (see Fig. 1 and X). Also, it was identified that the fermented products according to the present invention have an activity of decreasing the total number of bacteria in dirty water (see Fig. 3).

[53] Moreover, in one experiment, the effect of the products according to the invention on the bacterial flora in the intestine has been investigated (see Experimental Example 5). The results showed that the fermented products according to the present invention increase the amount of useful bacteria and decrease the amount of harmful bacteria, so do the probiotic activity (see Fig. 4).

[54] Also, in another experiment, the deodorant effects of the fermented products according to the present invention have been investigated (see Experimental Example 6). With the results, it has been identified that the fermented products have an activity of decomposing the ammonia/amines which are the causes of bad smells (see Fig. 5).

[55] Therefore, the fermented products according to the present invention have a low pH and a high content of lactobacillus and organic acid which have good acid-resistance
and bile acid-resistance, and have good antimicrobial activity, probiotic activity and
deodorant activity, so that the products can be used in various fields as an ingredient of
functional food composition, feed composition, antimicrobial composition and
deodorant composition. Above all, the low pH of the products facilitate the secretion of
digestive fluids and inhibit the growth of the harmful bacteria like Escherichia coli, the
high content of lactobacillus function as probiotics, and the organic acid have effects of
fatigue relief and so on.

As a result of analyzing the nutritive ingredients of the fermented products of the
present invention, the calories of those were 153.1 kcal/100g and the total content of
cellulose of those was 61.9%. From this, it could be said that they have low calories
and plenty of cellulose.

Therefore, the fermented products according to the present invention can be used as
diet food compositions, since they have low calories but the high quantity of insoluble
cellulose to give satisfied feelings.

As mentioned above, the fermented products according to the present invention can
be provided in the form of food composition. The food composition comprises all
kinds of functional food, nutritional supplement, health food, food additives and so on.
The food composition can be prepared to various types by the conventional preparation
methods. In particular, the fermented products according to the present invention can
be preferably provided in the form of functional cosmetic food, since the products have
specific flavors and tastes and excellent anti-oxidation effects.

More specifically, the fermented products according to the present invention can be
added into foods in the form of liquid or solid or can be ingested as they are. For
example, the liquid products can be produced in the form of tea, juice or drinks, and
the solid products can be ingested in the form of granulated, capsulated and powdered
things.

Also, the fermented products according to the present invention can be added into
beverages, fruits and their processed foods(for example, canned fruit, bottling, jam,
marmalade, etc.), fish, meat and their processed foods(for example, ham, sausage,
corned beef, etc.), breads and noodles(for example, udon, buckwheat noodle, instant
noodle, spaghetti, macaroni, etc.), fruit extracts, various drinks, cookies, wheat-gluten,
milk products(for example, butter, cheese, etc.), edible vegetable oil, margarine,
vegetable protein, retort foods, frozen foods, various seasonings(for example, soybean
paste, soy sauce, other sauce, etc.) and so on.

Moreover, the fermented products according to the present invention can be provided
as the antimicrobial composition. Preferably, the antimicrobial composition can be
used to inhibit the growth of pathogenic bacteria or kill those. The pathogenic bacteria
can be preferably Escherichia coli, Actinobacillus pleuropneumoniae, Salmonella and
so on, but it is not limited thereto.

[62] The antimicrobial composition can be prepared in the form of pharmaceutical agents which comprise the products according to the present invention alone or occasionally further at least one more pharmacologically acceptable carrier, excipient and diluent. As used herein, the term "pharmacologically acceptable" is understood that the object is physiologically allowable and it does not bring about allergy or any similar reactions when it is injected into human. The antimicrobial composition according to the present composition can be prepared as powder, granules, tablets, pills, sugarcoated pills, capsules, liquid medicine, gel, syrup, slurry, suspension, injection and so on by known method in related art.

[63] The effective dose of the present invention can vary depending on the adsorption rate, inactivity rate and evacuation rate of active component, age, sex and conditions of patients and the seriousness of disease, and it can be preferably 1-1000 mg/kg of weight/day.

[64] Moreover, the antimicrobial composition according to the present invention can be prepared in the form of a disinfectant, sterilization cleaner and food preservatives. The disinfectant can be prepared by diluting the fermented products according to the present invention with a diluent or a solvent for optimal concentration. The disinfectant can be used on the surface of organisms or the surface of inanimate matter, for example wood, metal, glass, ceramic, plastic, paper and clothes. The disinfectant can be applied to the surface of organisms or the surface of inanimate matter by dipping, using cotton buds, spraying and brushing. Preferably, the disinfectant can be used for the sterilization of a wound, the skin sterilization before surgery, the sterilization of surgery instruments, the sterilization of ducts, etc. at home or in an hospital.

[65] The sterilization cleaner can be prepared by the known method with at least one more excipients and additives which are commonly used in the cleaner manufacturing as well as the fermented products. The sterilization cleaner can be used for kitchen cleaning or food cleaning.

[66] The food preservative can be prepared with the fermented products in addition to the sitologically acceptable solvent or diluent to improve the conservativeness of foods. The food preservatives according to the present invention can be combined with the raw materials in manufacturing process of foods or can be added to foods in the form of the soluble suspension. Also, the foods can be dipped into the food preservatives according to the present invention or can be sprayed with the food preservatives.

[67] The fermented products can be provided in the form of the probiotic composition. As used herein, the term "probiotic activity" refers to the activity which inhibits the growth of harmful bacteria and accelerates the proliferation of useful bacteria. Hence, the probiotic composition according to the present invention can be used to prevent or
to treat for the intestinal diseases such as the infectious diarrhea by pathogenic microorganism (for example, Escherichia coli, Salmonella, Clostridium, etc.), gastroenteritis, inflammatory bowel diseases, neurological enteritis syndrome, microorganism overgrowth in small intestine, intestine acute diarrhea and so on.

The probiotic composition can be prepared and administered according to various known formulations and methods in related art. That is, the fermented products can be prepared in the form of tablets, capsules, suspension, syrup, wafer, antacid or granules by mixing with commonly used carriers in pharmaceutics field. Example of the proper carrier is a bonding agent, activator, disintegrant, excipient, solubilization agent, dispersing agent, stabilization agent, suspension agent, pigment, perfumes and so on. The probiotic composition can be preferably administered orally. The effective dose of the present invention can vary depending on the adsorption rate, inactivity rate and evacuation rate of active component, age, sex and conditions of patients and the seriousness of disease, and it can be preferably 1-1000 mg/kg of weight/day.

The fermented products can be provided in the form of the feed composition. The feed composition according to the present invention which is the liquefied fermented products can be provided to the animals with drinking water or feed. The feed can be the fermented feed, the assorted feed or the pellet type feed or silage, but it is not limited thereto.

The fermented products can be provided in the form of the deodorant composition. As used herein, the term "deodorant" refers to eliminating the bad smell. The deodorant composition can be prepared by adding purified water into the fermented products and diluting them appropriately.

Other advantages and characteristics of the invention will become more clearly apparent on reading the examples given below, which are given by way of examples and without implied limitation.

Brief Description of the Drawings

Figure 1 shows a graph describing the antimicrobial activity of fermented products of fruits or vegetables according to the present invention.

Figure 2 shows the antimicrobial activity of fermented products according to the present invention as compared with that of the positive control.

A: fermented products according to the present invention

B: Cetifour

C: Enrofloxacin

Figure 3 is a graph showing the effects of fermented products according to the present invention on the total number of bacterium of dirty water.
Figure 4 is a graph showing the effects of fermented products according to the present invention on the intestinal bacterial flora of the baby pigs.

Figure 5 is a graph showing the effects of fermented products according to the present invention on the decomposition of ammonia and amines which are the causes of bad smell.

Best Mode for Carrying Out the Invention

**<Experimental Example 1>**

Establishment of fermentation conditions for preparing the fermented products of fruits or vegetables according to the present invention

**<1-1>** Determination of injection orders of yeast and lactobacillus

It was determined that the injection orders of yeast and lactobacillus. For this end, the content of organic acid, the number of lactobacillus and the pH were measured in the fermented products in both cases of injecting with yeast and then lactobacillus and of injecting with lactobacillus and then yeast.

Apples, pears, bananas, oranges, grapes, persimmons and tomatoes at each 6 kg were prepared, washed with the detergent and then rinsed. The fruits were washed in the container with acetic acid and then rinsed again. In addition, green onions and onions at each 30g were prepared, washed and rinsed. The prepared fruits or vegetables were grinded and mixed. Water 27.94kg was added to the mixed fruits or vegetables 42.06kg. The sugar content was measured and regulated to concentration of 12 brix by adding brown sugar and confecting for about 30 minutes. The mixture was divided into two parts in which one part was injected with yeast(Saccharomyces cerevisiae KCCM 10714P) and the other part was injected with the mixture of Lactobacillus reutri(Lactobacillus reutri KCCM 10651P), Lactobacillus acidophilus(Lactobacillus acidophilus KCCM 10671P) and Lactobacillus brevis(Lactobacillus brevis KCCM 10714P), and they were cross-injected after 1 week.

In case of injecting with yeast, they were cultured at 25°C, and in case of injecting with lactobacillus, they were cultured at 35°C, since the activities of each of those are different according to the temperature. But the culturing period was 10 days each. The fermented products were compared with each other.

The pH was measured by the measuring instrument, and the number of lactobacillus was measured by plating the properly diluted fermented products over the Plate Count Agar with BCP(Brom Cresol Purple) which is lactobacillus analyzing medium and culturing the agar at 37°C for 18-36 hours and then counting the number of colonies. The content of organic acid was analyzed by gas chromatography by modifying the known method(The experimental method of the science of ruminant nutrition, Ha jong-

[89] The experimental results showed that it has a lower pH and a higher content of lactic acid and lactobacillus in case of the injection with yeast and then lactobacillus than the opposite case. In particular, the pH was relatively low but the number of alive lactobacillus was higher more than 10 times in case of the injection with yeast and then lactobacillus (see Table 1).

[90] Table 1

The pH, the content of lactic acid and the number of lactobacillus according to the injection order of yeast and lactobacillus

<table>
<thead>
<tr>
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<th>Injection with Lactobacillus after yeast</th>
<th>Injection with yeast after lactobacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>The content of lactic acid(%)</td>
<td>0.78</td>
<td>0.55</td>
</tr>
<tr>
<td>pH</td>
<td>3.2</td>
<td>4.3</td>
</tr>
<tr>
<td>The number of lactobacillus (CFU/g)</td>
<td>2.4 \times 10^7</td>
<td>1.8 \times 10^6</td>
</tr>
</tbody>
</table>

[91] <l-2> Determination of the period of fermentation

[92] To determine the optimal fermentation temperature and period, the fruit and vegetable mixtures which had been prepared by the same method as the Experimental Example 1 were injected and fermented with yeast(Sccharomyces cerevisiae KCCM 10714P) firstly and then he mixture of Lactobacillus reutri(Lactobacillus reutri KCCM 1065 IP), Lactobacillus acidophilus(Lactobacillus acidophilus KCCM 1067 IP) and Lactobacillus brevis(Lactobacillus brevis KCCM 10714P). The fermentation temperature was determined at 25°C in case of yeast and 35°C in case of lactobacillus, and the period of fermentation was changed respectively. After the fermentation completed, the number of yeast, the number of the lactobacillus, the pH and the content of organic acid were measured and compared. The number of yeast was
measured by plating the properly diluted fermented products over the yeast analysis medium (PDA, potato dextrose agar, Difco) and culturing this at 28°C for 3 days and then counting the number of colonies. The number of lactobacillus, the pH and the content of the organic acid were measured by the same methods in the Experimental Example <1-1>.

The experimental results showed that the number of yeast was the highest and the pH was the lowest in the eighth day of fermentation in case of yeast, and the number of the lactobacillus was the highest and the pH was the lowest and the content of organic acid was the highest in 12 day of fermentation in case of lactobacillus (see Table 2 and 3).

Table 2

<table>
<thead>
<tr>
<th>The first fermentation with yeast</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
<td>the period of fermentation (day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>the number of yeast (CFU/g)</td>
<td>* 10^6</td>
<td>* 10^6</td>
<td>* 10^8</td>
<td>* 10^7</td>
<td>* 10^6</td>
</tr>
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<td>3.8</td>
<td>3.2</td>
<td>3.3</td>
<td>3.2</td>
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</table>

Table 3

<table>
<thead>
<tr>
<th>The second fermentation with lactobacillus</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>the period of fermentation (day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>the number of lactobacillus</td>
<td>* 10^6</td>
<td>* 10^7</td>
<td>* 10^8</td>
<td>* 10^6</td>
<td>* 10^6</td>
</tr>
<tr>
<td>pH</td>
<td>3.5</td>
<td>3.4</td>
<td>3.2</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>the content of total organic acids (%)</td>
<td>5.6</td>
<td>6.8</td>
<td>7.4</td>
<td>7.2</td>
<td>7.2</td>
</tr>
</tbody>
</table>

<Example 1>

Preparation of fermented products of fruits or vegetables according to the present invention <1>
Apples, pears, bananas, oranges, grapes, persimmons and tomatoes at each 6 kg were prepared, washed with the detergent and then rinsed. The fruits were washed in the container with acetic acid and then rinsed again. In addition, green onions and onions at each 30g were prepared, washed and rinsed. The prepared fruits or vegetables were grinded and mixed. Water 27.94kg was added to the mixed fruits or vegetables 42.06kg. The sugar content of the mixture was measured and regulated to concentration of 12 brix by adding brown sugar and confecting for about 30 minutes. The mixture was injected with yedst (Saccharomyces cerevisiae KCCM 10714P) at 1% concentration and fermented at 25°C for 8 days firstly. Then, the first fermented products was injected with a mixture of Lactobacillus reuteri (Lactobacillus reuteri KCCM 10651P), Lactobacillus acidophilus (Lactobacillus acidophilus KCCM 10671P) and Lactobacillus brevis (Lactobacillus brevis KCCM 10714P) at 1% concentration, fermented and matured at 35°C for 12 days secondly.

Example 1
Preparation of fermented products injected and fermented with lactobacillus alone
The fermented products were prepared according to the same method as the Example 1, except that those were injected with lactobacillus only and the injection with yeast was omitted.

Example 2
Preparation of fermented products injected and fermented with yeast alone
The fermented products were prepared according to the same method as the Example 1, except that those were injected with yeast only and the injection with lactobacillus was omitted.

Experimental Example 2
Measurement of the content of physiologically active components of the fermented products according to the present invention
The contents of physiologically active components of the fermented products according to the present invention were measured and compared with those the fermented products which were injected and fermented with lactobacillus or yeast alone. That is, the pH, the content of organic acid or enzyme, the number of yeast, lactobacillus or Escherichia coli or the content of vitamin of the products according to Example 1 were compared with those of the products according to Comparative Example 1 and Comparative Example 2.

The pH, the content of organic acid, the number of yeast and the number of lactobacillus were measured according to the same method as the Experimental Example 1. The content of amylase among enzymes was measured by DNS (dinitrosalicylic acid) method (Miller, Anal. Chem., 31:426-428, 1959). In this method, the enzyme IU (unit) was defined as the amount of enzymes which can isolate 1 mol of the
products/min. Also, the activity of protease among enzymes was determined by measuring the tyrosine which was produced after the enzyme reaction using casein (0.7% in 20 mM tris-HCl buffer, pH 8.0) as a substrate with Folin method (Park, Thesis Collection of Graduate school Chun-Buk Univ 4:101, 1978). In this method, the enzyme 1U (unit) was also defined as the amount of enzymes which can isolate 1 mol of the products/min.

The number of Escherichia coli was measured by diluting the fermented products and adding them to the Escherichia coli analyzing medium (Chromocult coliform agar, Difco) and then counting the colonies after culturing them at 37°C for 18-36 hours. Vitamin B1 was measured by the test of microelements (Matsunaga et al. Analyst, chim. Acta. 98(25), 1978).

According to the experimental results, the fermented products of the present invention (Example 1) had a lower pH and a higher content of physiologically active components, that is organic acid, enzyme, lactobacillus and vitamin B1, as compared with the products which were injected and fermented with lactobacillus only (Comparative Example 1) or with yeast only (Comparative Example X). Particularly, the content of lactobacillus of the products according to the present invention was 10 times higher than those of the products in the Comparative Example 1 and 10,000 times higher than those of the products in the Comparative Example 2 (See Table 4).

Table 4
The contents of physiologically active components of the fermented products according to the present invention

<table>
<thead>
<tr>
<th>method of fermentation</th>
<th>example 1</th>
<th>comparative example 1</th>
<th>comparative example 2</th>
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</thead>
<tbody>
<tr>
<td>injection with yeast and lactobacillus</td>
<td>injection with lactobacillus alone</td>
<td>injection with yeast alone</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>3.2</td>
<td>4.5</td>
<td>3.3</td>
</tr>
<tr>
<td>enzymes</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lactobacillus</td>
<td>$10^7$</td>
<td>$10^6$</td>
<td>$10^3$</td>
</tr>
<tr>
<td>yeast</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>reducing sugar</td>
<td>51 69</td>
<td>45 22</td>
<td>48 24</td>
</tr>
<tr>
<td>vitamin B1</td>
<td>0.24</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>content of organic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactic acid(%)</td>
<td>0.78</td>
<td>0.27</td>
<td>0.34</td>
</tr>
<tr>
<td>malic acid(%)</td>
<td>7.09</td>
<td>3.12</td>
<td>3.57</td>
</tr>
<tr>
<td>acetic acid(%)</td>
<td>2.255 3</td>
<td>1.056 7</td>
<td>2.045 7</td>
</tr>
<tr>
<td>propionic acid (ppm)</td>
<td>128 4</td>
<td>50 0</td>
<td>55</td>
</tr>
</tbody>
</table>

[Table 4]

[115] ++: exist more than $10^5$
[116] +: exist more than $10^1$
[117] -: no exist
[118] <Experimental Example 3>
[119] Investigation of the acid-resistance and the bile tolerance of lactobacillus contained in the fermented products according to the present invention
[120] The acid-resistance and the bile tolerance of lactobacillus contained in the fermented products according to Example 1 were investigated. To analyze the acid-resistance and the bile tolerance of lactobacillus, the fermented products were diluted and injected to the MRS broth which is the lactobacillus culturing medium and cultured at 37°C for 20 hours.
The analysis of bile tolerance was carried according to the following method. The cultured lactobacillus was injected to MRS broth medium which contained 0%, 0.3% and 1% oxgall (Difco), at the viable count of 10⁶ cfu/ml, and then the number of lactobacillus was determined every hour while shaking culturing them at 37°C for 24 hours (Ahn et al., Kor. J. Anim. SciL 41(3), 335-342, 1999; B. Hyronimus et al., International Journal of Food Microbiology, 61, 193-197, 2000).

The analysis of acid-resistance was carried according to the following method. The cultured lactobacillus was injected to PDB (Potato Dextrose Broth) medium which was modulated to pH 2.5 and pH 3.0 with IN HCl, at the viable count of 10⁶ cfu/ml, and then the number of lactobacillus was determined every hour while shaking culturing them at 37°C.

According to the experimental results related to the bile tolerance, the lactobacillus presented in the fermented products according to the invention showed the survival rate 138% after 24 hours, even in the concentration of oxgall of 1%. Moreover, as the oxgall concentration increases, the survival rate of the lactobacillus became increased. It was identified that the lactobacillus has the excellent bile tolerance (see Table 5).

Table 5

<table>
<thead>
<tr>
<th>concentration of oxgall(%)</th>
<th>0 hour</th>
<th>12 hours</th>
<th>24 hours</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>118</td>
<td>122</td>
</tr>
<tr>
<td>0.3</td>
<td>100</td>
<td>129</td>
<td>131</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>137</td>
<td>138</td>
</tr>
</tbody>
</table>

Also, according to the experimental results related to the acid-resistance, the lactobacillus showed the survival rate of 100% and 83% after 12 hours in medium modulated to pH 3.0 and 2.5 respectively. It was identified that the lactobacillus have the excellent acid-resistance (see Table 6).
[Table 6]

The survival rate of lactobacillus according to the pH (%)

<table>
<thead>
<tr>
<th>pH/hour</th>
<th>0 hour</th>
<th>1 hour</th>
<th>3 hours</th>
<th>12 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>100</td>
<td>96</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td>3.0</td>
<td>100</td>
<td>98</td>
<td>91</td>
<td>100</td>
</tr>
</tbody>
</table>

[127] <Experimental Example 4>

Investigation of antimicrobial activity of the fermented products according to the present invention

The antimicrobial activity of the fermented products according to Example 1 was investigated. For this end, the fermented products were centrifuged at 4000rpm for 10 minutes, and the supernatants were filtered at the level of 0.45D. And then the sterile filter disk (semidiameter 5mm) with the filtrate was arranged in MRS medium, and injected with Actinobacillus pleuropneumoniae (Actinobacillus pleuropneumoniae ATCC 55454), Salmonella (strains of gallinarum ATCC 700623), intestinal toxigenic Escherichia coli of the suckling baby pig and the weaning baby pig (E. coli ATCC 25922) and intestinal pathogenic Escherichia coli of the weaning baby pig (E. coli ATCC 49105) respectively, 30 minutes later. In case of Actinobacillus pleuropneumoniae, casman(Difco) medium containing NAD (nicotinamide adenine dinucleotide) was used. At 6, 12, 24, 36 hours after injecting with each bacteria, the zone of inhibition was measured by the standard yardstick and observed the semidiameter of clear zone in which the bacteria do not grow up. Also, the antibiotics like Cetifour and Enrofloxacin were used as positive controls. The two sterile filter disks per each bacteria were investigated in two times and the zone of inhibition was presented as a mean value standard deviation. Also, the difference between the zones of inhibition of each bacterium was compared by the Student's t test.

Moreover, the effect of the products on the total content of germs in the dirty water was investigated. To do this, the dirty water of the cattle shad was plated over LB agar medium(Difco) and cultured at 37°C for 16-20 hours to produce colonies. The fermented products diluted at 1% were sprayed on the solid culture medium every 3
days for 9 days, and the total numbers of bacteria were compared by identifying the numbers of colonies before and after spraying the fermented products.

According to the experimental results, the fermented products according to the present invention showed the highest antimicrobial activity against *Actinobacillus pleuropneumoniae*, and showed the antimicrobial activity also against *Escherichia coli* and *Salmonella* (see Fig. 1). In addition, the antimicrobial activity of the products against *Actinobacillus pleuropneumoniae* was maintained in a regular level without large loss from 12 hours later. The antimicrobial activity of the products against other bacteria reached pick at 6 hours later and decreased gradually since then. The antimicrobial activity of the fermented products according to the present invention is similar to that of the antibiotics like Cetifour and Enofloxacin (see Fig. 2).

Moreover, it was identified that the fermented products according to the invention have the activity of inhibiting the growth of bacteria contained in dirty water (see Fig. 3).

**<Experimental Example 5>**

The effect of the fermented products according to the present invention on the bacterial flora within the intestines

After administering three-week-old baby pig with the drinking water containing the fermented products according to Example 1 at 1% by weight for 5 weeks, it was investigated that the changes of the bacterial flora of baby pig according to the provision of the products by feces sampling. The feces sampling for the analysis of the bacterial flora within the intestines was implemented by taking fresh feces (taken before falling on the floor), putting them into the sampling tube promptly, sealing the tube and analyzing the sample promptly in the laboratory.

The analysis of the bacterial flora within the intestines was carried by the following method. Lactobacillus, yeast, salmonella, *Escherichia coli* in the feces were investigated respectively. The medium used for analysis was the Plate Count Agar with Brom Cresol Purple (Eiken, Japan) for lactobacillus, PDA (potato dextrose agar, Difco) for yeast, XLT-4 Agar (Difco) for salmonella and Chromocult Agar (Difco) for *Escherichia coli* respectively. The feces sample 1g in the 50 mL tube was added with saline solution 9 mL and extracted for 30 minutes in the room temperature. The extracted solution 1 mL was diluted in stages, plated over the analyzing medium and cultured, and the colonies were counted. Yeast was cultured at 28°C for 2 days, and the rest of bacteria were cultured at 37°C for 18-36 hours.

The experimental results showed that the number of beneficial lactobacillus was increased and the numbers of harmful *Escherichia coli* and salmonella were decreased in case of being provided with the fermented products according to the present invention as compared with that before the administering (see Fig. 4).
**<Experimental Example 6>**

Investigation of deodorant activities of the fermented products according to the present invention.

The deodorant activities of the fermented products according to the present invention were investigated. To this end, after spraying the fermented products of Example 2 diluted at 100 times on the area of waste matter flowing at the predetermined time, the content of ammonia/amines which are the causes of bad smell was measured at the predetermined time in the morning, that is just before of ventilation, inside of the farm. The measurement of ammonia/amine gas inside of the farm was implemented by each detector tube of Gastec(Gastec, model 801, Japan). As the detector tube, Gastec Detector tube no.3L was used for detecting the ammonia, and Gastec Detector tube no.180 was used for detecting the amine gas. The gas was taken at a height of 10 cm from the floor which is the height of nose of the animal (pig). The experiment was carried for 6 weeks.

The experimental results showed that ammonia and amine were decreased gradually with time and became about 1/7 level of the first level after 6 weeks (see Fig.5).

**<Example 2>**

Preparation of fermented products of fruits or vegetables according to the present invention <2>

The fermented products prepared by the method of the Example 1 were extracted and separated to solid and juice. The solid was freeze-dried at -40°C and grinded at 100 mesh to make powder. The separated liquid was spary-dried and processed to powder.

**<Example 3>**

Preparation of fermented products of fruits or vegetables according to the present invention <3>

The fermented products were produced by the same method as Example 2, except that the solid was hot-air dried at less than 40°C.

**<Experimental Example 7>**

Measurement of the content of physiologically active components according to the drying methods.

The pH and the contents of enzyme, lactobacillus, yeast and Escherichia coli were measured by the same method as the Experimental Example 2 for the fermented products processed by freeze-drying in Example 2 and the products processed by hot-air drying in Example 3.

According to experimental results, the contents of enzyme and lactobacillus were higher in the products freeze-dried(Example 2) than in the products hot-air dried(Example 3). In particular, the content of lactobacillus in the products freeze-dried was 100 times higher than that of the products hot-air dried (see Table 7).
The contents of physiologically active components in the fermented products according to the different drying methods

<table>
<thead>
<tr>
<th></th>
<th>example 2</th>
<th>example 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>drying method</td>
<td>freeze-drying</td>
<td>hot-air drying</td>
</tr>
<tr>
<td>pH</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>enzyme</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>lactobacillus</td>
<td>$10^7$</td>
<td>$10^8$</td>
</tr>
<tr>
<td>yeast</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**<Experimental Example 8>**

Investigation of the nutrition ingredients of the fermented products according to the present invention

The calories, carbohydrate, coarse protein, coarse fat, moisture, ash, sodium and dietary fiber were measured for the fermented products processed by hot-air drying in Example 3 by the known methods (Chae, soo kyu, standard food analysis, Gigoo culture press, p207-409, 1998).

According to experimental results, the fermented products presented the calories of 153.1 kcal per 100g and the total content of dietary fiber of 61.9%. So, it is known that the products have low calories and plenty of dietary fiber (see Table 8).
The nutrition ingredients of the fermented products according to the present invention

<table>
<thead>
<tr>
<th>Analysis Item</th>
<th>Analysis Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (Kcal/100g)</td>
<td>153.1</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>18.1 (except dietary fiber 61.9%)</td>
</tr>
<tr>
<td>Coarse protein (%)</td>
<td>6.9</td>
</tr>
<tr>
<td>Coarse fat (%)</td>
<td>5.9</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>5.6</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.6</td>
</tr>
<tr>
<td>Sodium (mg/100g)</td>
<td>10.5</td>
</tr>
<tr>
<td>Soluble dietary fiber (%)</td>
<td>7.5</td>
</tr>
<tr>
<td>Insoluble dietary fiber (%)</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Example 4

Preparation of fermented products of fruits or vegetables according to the present invention

The fermented products were produced by the same method as Example 1, except that fruits such as kiwi fruits, pineapples or strawberries and vegetables such as tomatoes, cabbages, kales, carrots or broccolis were mixed-used at the same amount (each 6 kgs), and green onions and onions were added to the mixture at each 5% (30g).

Industrial Applicability

As described above, the fermented products prepared according to the present invention are excellent in the antimicrobial activity, the probiotic activity and the deodorant activity, comprising a high content of lactobacillus which have good acid-resistance and bile tolerance and physiologically active components which are beneficial to the human body such as organic acids, enzymes or vitamins in great quantities, as compared with the products injected with yeast or lactobacillus alone. Moreover, the fermented products according to the present invention have low calories and high dietary fiber content. Therefore, the fermented products according to the
present invention are very useful as an ingredient of functional food, diet food, an-
timicrobial, probiotic and feed composition.

[164]
Claims

[I] A preparation method of fermented products of fruits or vegetables, comprising of the steps:
(a) grinding fruits, vegetables or the mixtures thereof, and then mixing them with purified water and sugar;
(b) injecting and fermenting the mixture of step (a) with yeast; and
(c) injecting and fermenting the fermented products of step (b) with lactobacillus.

[2] The method of claim 1, the fruits of step (a) are selected from the group consisting of apples, pears, oranges, bananas, persimmons, grapes, kiwis, pineapples and strawberries.

[3] The method of claim 1, the vegetables of step (a) are selected from the group consisting of tomatoes, cabbage, kale, carrots, broccoli, green onions and onions.

[4] The method of claim 1, the purified water of step (a) is added in an amount of 60-65% by weight based on the weight of the fruits, the vegetables or the mixtures.

[5] The method of claim 1, the sugar is added to the fruits, vegetables or the mixtures for the sugar content to be 10-14 brix in step (a).

[6] The method of claim 1, the yeast of step (b) is Saccharomyces cerevisiae or Saccharomycopsis fibuligera.

[7] The method of claim 1, the fermenting of step (b) is performed at 24-26 °C for 6-10 days.

[8] The method of claim 1, the lactobacillus of step (c) is selected from the group consisting of Lactobacillus reutri, Lactobacillus acidophilus and Lactobacillus brevis.

[9] The method of claim 1, the fermenting of step (c) is performed at 33-37 °C for 10-14 days.

[10] The method of claim 1, further comprising step of extracting juice from the fermented products of step (c) and separating the juice and solid.

[II] The method of claim 10, further comprising step of spray-drying the extracted juice.

[12] The method of claim 10, further comprising step of freeze-drying or hot-air drying the separated solid.


[14] Fermented products of fruits or vegetables prepared by the method according to any one of claims 1 to 13.

[15] The fermented products of claim 14, which are powder.

Fig. 1

Fig. 2

Toxigenic E.coli  Salmonella  Actinobacillus pleuropneumoniae
Fig. 3

Total bacteria before the application of the fermented fruits and vegetables solution

Total bacteria after 5 days of the application of the fermented fruits and vegetables solution

Fig. 4

Days of provision

Before provision 1 week 2 weeks 3 weeks 4 weeks 5 weeks

Number of microorganisms (log 10/cell)

Substitute sheet (Rule 26)
Fig. 5

- Ammonia
- Amines
A. CLASSIFICATION OF SUBJECT MATTER

A23L 1/212(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 A23, 1/212, 2/02, 2/38, 2/68, C12G 3/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Utility models and applications for Utility models since 1975

Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKIPASS, Delphion, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>A</td>
<td>WO 92/13939 A1 (BARRE PHILIPPE et al) 20 August 1992 see abstract</td>
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* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

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See patent family annex

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"&" document member of the same patent family

Date of the actual completion of the international search

25 OCTOBER 2007 (25.10.2007)

Date of mailing of the international search report

25 OCTOBER 2007 (25.10.2007)

Name and mailing address of the ISA/KR

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Facsimile No 82-42-472-7140

Authorized officer

KIM, Jae Hyun

Telephone No 82-42-481-5626

Form PCT/ISA/210 (second sheet) (April 2007)
## INTERNATIONAL SEARCH REPORT

### Information on patent family members

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