Welcome to Japan! Welcome to Tokyo!

Recepción a Japón !!

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The Novel Hair Dyeing Technology by Using Melanin Precursor Prepared by an Aspergillus Tyrosinase

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1. Background of Our Hair Research

Relationship between melanin and color of the hair

- Melanin type and amounts affect color and brightness of the hair -

<table>
<thead>
<tr>
<th>Melanin Type</th>
<th>Hair Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pheomelanin Rich</td>
<td>Red, Blond</td>
</tr>
<tr>
<td>Eumelanin Rich</td>
<td>Black, Brown</td>
</tr>
</tbody>
</table>

Asian hair has more Eumelanin
Relationship between melanin and color of the hair

- Melanin type and amounts affect color and brightness of the hair -

<table>
<thead>
<tr>
<th>Less Melanin</th>
<th>Bright</th>
<th>Dark</th>
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Asian hair has more Eumelanin
- Melanin type and amounts affect color and brightness of the hair -

Asian hair has more Eumelanin

Eumelanin Rich

Brown
Blond
Black
Red

Pheomelanin Rich

More Melanin

More

Bright

Less Melanin

Dark

Less
The difference between Gray and Black Hair

- The difference is only melanin -

Male hair sample from a Japanese (50’s)

Gray hair

No melanin

Black hair

Melanin Granules
• Eumelanin is a polymer made from Tyrosine or DOPA.
• This pathway is initiated by Tyrosinase oxidation reaction.
• Melanin precursors are converted to Melanin by Oxygen.

Melanogenesis  Eumelanin biosynthesis

- Eumelanin is a polymer made from Tyrosine or DOPA.
- This pathway is initiated by Tyrosinase oxidation reaction.
- **Melanin precursors** are converted to Melanin by Oxygen.

A new idea for hair dyeing system with melanin (precursor)

**Melanin precursor**

Into Hair (Cuticle)

O₂ (Air)

**Melanin**

**Benefits**

- Melanin and its precursors are **biological substances**.
- The Melanin precursor is small enough to penetrate hair.
- It converts to melanin by exposure to air.
- Melanin has a **natural** dark color.

**Issues**: The Melanin precursor is very unstable. None of the natural compounds were industrially available.
By the way, we have traditional biotechnology in Japan.

Fungus and yeast have been used in food manufacturing and various fermentation processes in Japan for over 1000 years.

02. Preparation of Melanin Precursor using fungus Tyrosinase

Manufacturing new ingredient from natural resources using same process as in nature.

Collaboration between Kao & Gekkeikan
Japanese Sake manufacturing

Japanese Traditional Biotechnology

Rice

Clean Water

Saccharomyces cerevisiae (yeast)

Aspergillus oryzae (fungas)

酒造り (SAKE Dukuri)
Alcohol Fermentation

Traditional Brand from 17th century (400 yrs)
There was a color problem, occasionally, during 1950s and 1960s.
As a result, the Black Koji was identified, and the Melanin formation mechanism was found.

Tyrosinase from *Aspergillus oryzae* makes melanin.

Tyrosinase was found to be expressed in Black Koji.

The Japanese Sake company found a tyrosinase strongly expressed in Black Koji.

Koji: Fermented rice with Aspergillus oryzae

New Collaboration research was started

• Eumelanin is a polymer made from Tyrosine or DOPA.
• This pathway is initiated by Tyrosinase oxidation reaction.
• Melanin precursors are converted to Melanin by Oxygen.

Which should be produced?

Melanin precursors

- DOPA
- Tyrosine
- Dopaquinone
- Cyclodopa (DHICA)

Eumelanin

- 5,6-Dihydroxyindole
- 5,6-Dihydroxyindole-2-carboxylic acid
The dyeing ability of melanin precursors

DHICA: 5,6-Dihydroxyindline 2-carboxylic acid
DHIoleCA: 5,6-Dihydroxyindole 2-carboxylic acid
DHI: 5,6-Dihydroxyindole (DHI)
Dyeing condition: 3% Ammonia, 0.1% each compound, 3 times
• Tyrosinase is produced by fermentation.

• Dopa is converted to Dihydroxyindole in tyrosinase process.

• The product is adjusted for cosmetics materials.
Melanin precursor was produced from Plant-originated Dopa.

- DOPA (the starting material) is of Plant-origin.
- Melanin precursor was produced by using the same process as in nature.
- The Melanin precursor is a sustainable compound.
03. Application of Melanin Precursor to Hair Dye

Development of a natural coloring product for gray hair.
New Concept of Natural Hair Dye

• The new dyestuff, 5,6-dihydroxyindole, has been manufactured from natural resources using a similar process to nature.

• It ensures “True Natural Hair Color” by recovering color by melanin.

• Air oxidation system without H₂O₂
  – Gentle to hair: less damage
  – Easy to use: no mixing, no long waiting.
**Formulation of the Hair Dye**

**Foam type:** Ease of Use  
**Aerosol:** maintain stability by Oxygen shutoff

- Dihydroxyindole (New Dyestuff)
- Foamer (surfactant)
- Alkali
- Solvents
- Antioxidants
- Thickeners

**How to Use:** 20g of the product was applied to hair. After standing for 5 min. hair is washed with shampoo.
The relationship between DHI conc. and Dyeing ability

DHI at 0.1 - 0.3% dyed gray hair **gradually**.

DHI: 0.1% or 0.3% + 3% ammonia

Procedure: Chinese grey hair, RT, 5min, shampooed and dried,
This treatment was repeated as indicated.
pH 10 with ethanolamine was most effective

Melanin precursor in the formulation was stable for 6 months
Brown and Ash shades were achieved.

Brown shade

Ash shade

DHI: 0.3% + 1% Ethanolamine + Antioxidants

Procedure: Chinese grey hair, RT, 5min, shampooed and dried,
This treatment was repeated up to 5 times.
Melanin precursor is converted to Melanin by air.

Dyeing ability: ±

5 min just after discharging.

Dyeing ability: ++ Easy to use: no mixing, no long waiting period.
1. Melanin precursor can penetrate into the cuticle of hair.
2. Melanin is formed by exposure to air.
3. Gray hair is gradually dyed by step-wise use (3-5 times).
4. The final color of hair then looks more natural.

How to Use
- Treatment
- 5 min.
- Shampoo
Panelist results

- Each repeated treatment brought **natural change**.
- The color of hair became very natural.
- The color only faded after application was stopped.
Advantages of the new technology

Hair damage level

<table>
<thead>
<tr>
<th>Cysteic acid [mol%]</th>
</tr>
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<tbody>
<tr>
<td>Non Treated</td>
</tr>
<tr>
<td>New Technology</td>
</tr>
<tr>
<td>Hair Dye</td>
</tr>
<tr>
<td>Direct Dye</td>
</tr>
</tbody>
</table>

Skin staining

Skin → 30 min. → Washing with water

Hair → Hydrolysis → A.A. analysis (HPLC)
A New Natural Hair Dye

• The new dyestuff from natural resources
  – DHI has been manufactured using a similar process to nature.

• Restoring Melanin into gray hair.
  – Recovery of natural color by melanin

• Gentle to hair and Easy to use
  – Air oxidation system without $\text{H}_2\text{O}_2$
Gracias mucho!!

Thank you for your attention!
Gracias mucho!!
Argentina!