

## Effects of astaxanthin from *Haematococcus pluvialis* on human skin

- Patch test; Skin repeated application test; Effect on wrinkle reduction -

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**Abstract:** Astaxanthin is a natural color carotenoid found in salmon, salmon eggs, krill, and crab. Therefore, astaxanthin has been contained in the human diet for a long time. Astaxanthin from krill has been used for cosmetics to suppress post-UVB hyperpigmentation in human skin and food color additives. Recently, astaxanthin from *Haematococcus pluvialis* is available using new fermentation technology of *H. pluvialis* and it is used for dietary supplements, food color additives and cosmetics.

Effects of astaxanthin from *Haematococcus pluvialis* on human subjects were tested. No serious adverse effects were observed by patch testing and sequencing applied test on human skin. In a pilot study, the skin repeated application test of cream containing astaxanthin on human skin showed the visual wrinkle reduction. The present paper described about patch testing, skin repeated application test, and a pilot study evaluating the wrinkle reduction effect on human skin.

**Key words:** astaxanthin, patch testing, skin repeated application test, wrinkle reduction, *Haematococcus pluvialis*

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## 1. Introduction

Astaxanthin belongs to the carotenoid family that includes  $\beta$ -carotene etc. It is a red color widely and naturally distributed in foods such as fishes e.g. salmon, sea bream etc. and *Crustaceans* such as shrimps, crabs etc. which have been well consumed. In the past, astaxanthin from krill was only put into practical use. However, astaxanthin from *Haematococcus pluvialis*, a green algae, is able to accumulate sufficient quantities and economically after establishment of its industrial cultivating technology and the compound expects to increase its number of potential applications.

Astaxanthin from *Haematococcus pluvialis* has been listed in the Standards of Cosmetic Combination Ingredients by Category<sup>2)</sup> and both are put into practical use.

Recently, strong anti-oxidative action<sup>3)4)</sup> of astaxanthin was reported and diverse range of its physiological functions and bioactivities have been studied intensely. For instance, many papers on its actions, such as anti-inflammatory action<sup>5)</sup>, anti-arteriosclerotic action<sup>6)7)</sup>, action against diabetes<sup>8)9)</sup>, daily rhythm regulating action<sup>10)</sup>, immuno-activating action<sup>11)</sup>, anti-stress action<sup>12)</sup> and inhibition of urinary bladder carcinogenesis<sup>13)</sup> etc., have been reported. On the other hand, its actions on skin, such as inhibition of pigmentation<sup>14)</sup>, inhibition of melanin-generation and of light-induced aging<sup>15)</sup> have been reported.

At this time, we prepared a cream preparation combined with astaxanthin from *Haematococcus pluvialis*, which may be expected its cosmetic effect for the skin and performed a patch test and a skin repeated application test in human using the preparation in order to assess safety on the skin. At the same time, we examined its effect on skin moisture retention as well as on skin condition. We will report the results as follows:

## 2. Patch Test in Human Subjects

We performed a patch test using human subjects in order to assess safety on the skin, using a preparation of astaxanthin from *Haematococcus pluvialis* and a cream preparation combined with astaxanthin from *Haematococcus pluvialis*.

### 2-1. Test materials

Sample No.1: Astaxanthin from *Haematococcus pluvialis*

AstaReal Oil 50 FR was used as Sample No.1: Dried powders, obtained from the disrupted cell-walls of *Haematococcus pluvialis* supplied by Microgaia Inc. (Maui Island of Hawaii State, USA) were extracted with acetone, and then tri (capryl / capric acid) glycerol was added to the

extracts to make 5% (as free astaxanthin) of astaxanthin preparation.

Sample No.2: Cream base (Control)

Sample No.3: Cream base (Control) + astaxanthin

The astaxanthin from *Haematococcus pluvialis* of Sample No.1 and the cream base of Sample No. 2 were combined to form 0.7 mg/g of astaxanthin cream.

## 2-2. Subjects

The study was performed using 45 subjects of healthy adults who consented to be enrolled in the study. Table 1 shows an age structure.

**Table 1. Age structure of subjects in Patch Testing**

| Age          | Number of subjects |        |
|--------------|--------------------|--------|
|              | Male               | Female |
| 21-25 yrs.   | 12                 | 28     |
| 26-30 yrs.   | 2                  | 1      |
| 31 – 35 yrs. | 0                  | 0      |
| 35 – 40 yrs. | 0                  | 2      |
| Total        | 14                 | 31     |

## 2-3. Method

Samples were applied on the medial site of the upper arm subjects and a plaster (Fin-Chamber, Taisho Pharmaceutical Co., Ltd.) was placed over the treated area. The plaster was removed after 24 hours and then the skin-irritability observed after 30 minutes (24 hours) and also a further 24 hours after the removal (total 48 hours).

## 2-4. Judgement

Judgment was conducted in accordance with “Standard of Japanese Patch Testing Research Group” and evaluation of each Sample was made by calculation of skin-irritability index in accordance with Evaluation Criteria of Skin-Irritability Index<sup>16)</sup>.

## 2-5. Results

- 1) Astaxanthin from *Haematococcus pluvialis* (Sample 1): 42 subjects showed no reaction (-) to the applied AstaReal® oil after 24 and 48 hours. Of the three subjects only 2 (Male 24 yrs. and Male 25 yrs.) showed slight reactions ( $\pm$ ) after 24 hours, and one subject (Female 25 yrs.) reacted ( $\pm$ ) only after 48 hours. The male subjects (Male 24 yrs., Male 25 yrs.) experienced dermatitis that was induced by the adhesive plaster in contact with the skin and suspecting its expansion to the tested sites. Overall Skin-Irritability Index showed 3.3.

- 2) Cream base (Sample No.2): 42 subjects experienced no skin reactions to the cream base control. Only 2 (Male 24 yrs., Male 25 yrs.) of 45 subjects showed slight signs of irritation ( $\pm$ ) only after 24 hours, and one subject (Female 25 yrs.) showed  $\pm$  only after 48 hours. Of the two male subjects (Male 24 yrs., Male 25 yrs.), dermatitis was again induced by adhesive plaster, suspecting its expansion to the tested sites. Skin-Irritability Overall Skin Irritability Index showed 3.3.
- 3) Cream base + astaxanthin (Sample No.3): 43 of 45 subjects showed no reaction (-) to the astaxanthin containing cream. Only 2 (Male 24 yrs., Male 25 yrs.) of 45 subjects showed signs of irritation ( $\pm$ ) both after 24 and 48 hours. The both had remarkable dermatitis induced by adhesive plaster and suspected expansion of the dermatitis to the tested sites. Overall Skin-Irritability Index showed 2.2.

## 2-6. Conclusion

All the three Samples were judged safe and concluded no problem in safety concerning primary irritant induction.

## 3. Skin Repeated Application Test in Human Subjects

Since the safety for skin-irritability was assessed in Patch Test using the cream preparation combined with astaxanthin from *Haematococcus pluvialis*, we performed a skin repeated application test using the cream preparation in order to assess safety as well as to evaluate its effects on human skin.

### 3-1. Test material

The cream preparation of Sample No.3 (see paragraph 2-1) was used.

### 3-2. Subjects

The study was performed using 11 subjects of healthy female adults who consented to be enrolled in the study. Table 2 shows an age structure.

**Table 2. Age structure of subjects in Skin *Repeated* Application Test**

| Age Group | Number of subjects |
|-----------|--------------------|
| 20's      | 3                  |
| 30's      | 1                  |
| 40's      | 6                  |
| 50's      | 1                  |
| Total     | 11                 |

### **3-3. Method and study period**

After washing face and applying cosmetic lotion on the face every morning and evening, the subjects applying a small bean sized volume (approx. 0.2 g) of sample on their face and spread it thinly from under the eyes to the cheeks. They were permitted to use other cosmetics likely as usual. Study period was 3 weeks-with daily applications.

### **3-4 Test Parameters**

Visual examination of the following measurements were performed before and after the 3-week application respectively. They were performed in a room kept approx. 21°C- R.T. and 55 % - R.H., after 15 minutes of face washing.

#### **3-4-1. Visual Inspections for Safety.**

Irritability to skin and safety were evaluated observing subjects' face-skin condition before and after the application.

#### **3-4-2. Skin moisture content**

Skin moisture content of the outer site, approx. 1cm from the left outer canthus was measured using Moisture Checker MY707S, Scalla Inc.

#### **3-4-3. Questionnaires**

Subjective symptoms on skin condition of enrolled subjects in this study were checked by questionnaires, and safety and effectiveness of the cream preparation were evaluated.

### **3-5. Results**

#### **3-5-1. Safety assessment**

There were no subjects that showed deterioration in skin condition caused by the test samples.

#### **3-5-2. Clinical efficacy**

8 subjects out of 11 were observed improvement of subjective symptoms in skin-dryness. One subject with senile pigment macule and 2 subjects with chloasma (sun-burn spots) were observed light improvement in skin-colour.

#### **3-5-3. Skin moisture content**

**Fig.1** shows changes of skin moisture content of the outer site, approx. 15 cm from the left outer canthus before and after the application respectively. A difference, + 3.32% between the value before application (mean value of moisture content: 43.059%) and the value after application (mean value of moisture content: 46.381%) showed an elevation of moisture content

significantly in accordance with a paired T-test ( $P < 0.05$ ).

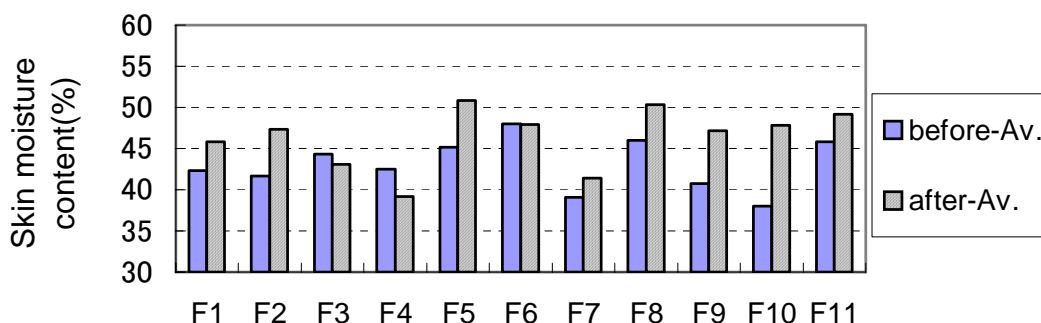


Fig.1. Moisture retention effect after 3 weeks-application of Astaxanthin Combined Cream

### 3-5-4. Questionnaire Responses

Fig.2 shows skin condition obtained from subjective symptoms on skin by questionnaires before the application.

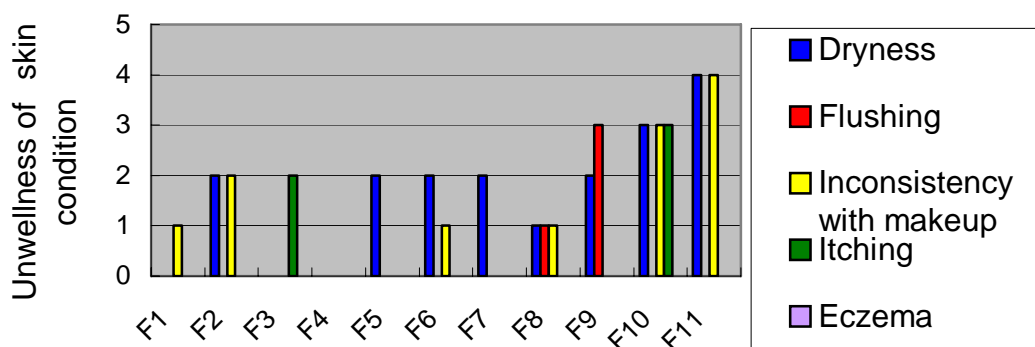


Fig.2. Subjective symptoms on skin before application of Astaxanthin combined cream.

Fig.3 shows the results of safety assessment by questionnaire after 3 weeks repeated application. Almost of all subjects felt slight improvements with either “Dryness”, “Flushing”, “Itching”, “Inconsistency with make-up” or “Eczema”. Definitive improvements in “Dryness” and “Inconsistency with makeup” were observed.

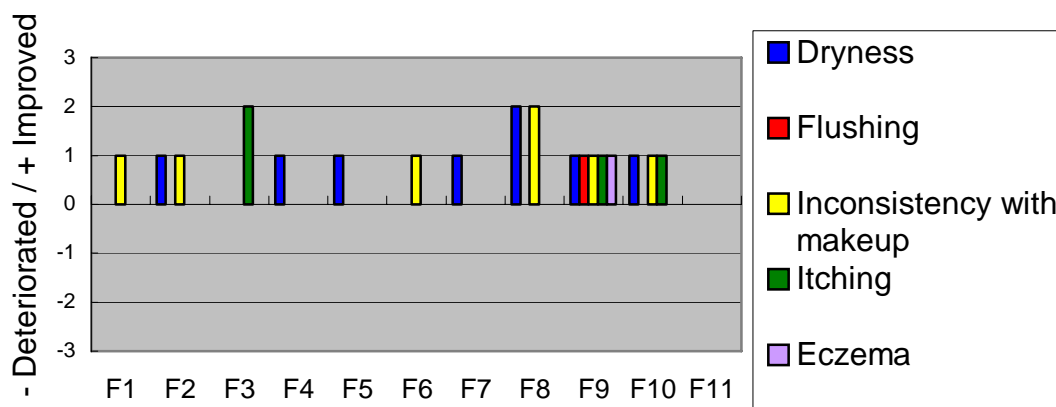


Fig.3. Safety evaluation in improvement of skin condition after a 3-week application of Astaxanthin combined Cream

Fig.4 shows the improving effects by questionnaires after 3 week-repeated applications. Almost of all subjects obtained improvement of skin condition except one subject (Female 29 yrs.) who had a feeling of coarse skin.

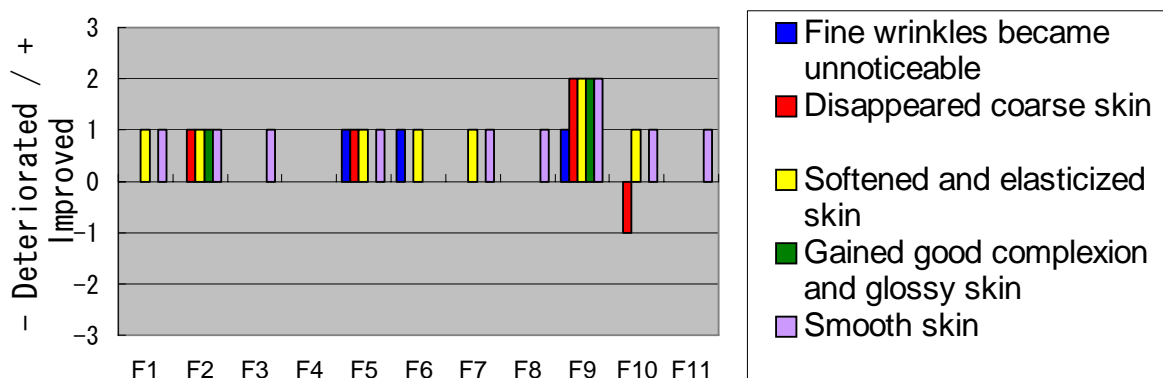


Fig.4. Efficacy evaluation in improvement of skin condition after 3 weeks application of Astaxanthin Combined Cream

### 3-6. Conclusion

No skin-irritability nor deterioration of skin condition was observed after 3 week-application of the cream preparation combined with astaxanthin and the same result in safety assessment was also obtained in this Skin Repeated Application Test as in the preceded Patch Test. Furthermore, the skin moisture retention increased significantly and improvement of skin condition was demonstrated in this study.

## 4. Beauty Effect

From the results showing increase of skin moisture retentivity in Skin

Repeated Application Test, we have expected a beauty effect of the cosmetic preparation combined with astaxanthin. Then, we performed a preliminary study on beauty effect of astaxanthin using 3 subjects.

**4-1. Test material**

The cream preparation of Sample No. 3 (See Paragraph 2-1) was used.

**4-2. Subjects**

The study was performed using 3 subjects of healthy female adults who consented to be enrolled in the study. Table 3 indicates an age structure and skin-types.

Table 3. Age structure and skin-types of subjects in the preliminary study on beauty effect

| Subject | Age Group | Skin-type   |
|---------|-----------|-------------|
| M1      | 40's      | Normal skin |
| M2      | 30's      | Dried skin  |
| M3      | 30's      | Mixed type  |

**4-3. Method and study period**

Study period: 2 weeks

Method: After washing face, apply a test lotion on the whole face, and then paint the test cream of Sample No. 3 spreading from under the eyes to the cheeks. Other cosmetics are permitted to use likely as usual.

**4-4. Condition of observation**

Observation was performed after 15 minutes under keeping rest with sitting position after face-washing in an environmental test room maintained 20°C-R.T. and 65%-R.H.

**4-5. Test parameters**

Questionnaire: Feeling of use / Skin condition/ Daily skin diary/ Inspection / Palpation

Measurement of skin moisture content: Measured at the both sites, left and right outer canthi and cheeks.

Measurement of sebum content: Measured sebum of forehead and cheeks using transmission sebum-meter

Skin wrinkle-coefficient of outer canthi / Skin grain-coefficient of cheeks: Input images using Skin Surface Image Analyzer and verted thickness (deepness) / length of wrinkles and size / Uniformity of skin-grain into numerical values.



Magnified skin photograph: Recorded using Medical Nikkol (Nikon) and Microscope (x60)(Hirocks).

#### **4-6. Results**

##### **4-6-1. Skin condition evaluated by subject's self-assessment**

**Fig.5** shows skin condition in each item evaluated by subject's self-assessment (See Attachment 1).

##### **4-6-2. Inspection / Palpation**

**Fig.6** shows the results obtained from skin-inspection and palpation by beauty specialists. Moderate improvement of "Fine wrinkles" and "Flabbiness under eyes" which are typically caused by skin-dryness was observed in **M2** (Dried skin) and **M3** (Mixed type). The skin conditions expressed as "Moistness", "Smoothness" and "Elasticity" obtained from skin-palpation showed improving tendency in all cases (See Attachment II).

##### **4-6-3. Skin moisture content / sebum (skin oil) content**

**Fig.7** shows changes of skin-moisture content and sebum (skin oil) content after 2 week-application of the cosmetic preparation combined with astaxanthin. Elevations of skin moisture content were observed in the subjects, **M2** (Dried skin) and **M3** (Mixed type). It was considered that the sample had moisturizing effects. Increased sebum content was observed in **M1** (Normal skin) and **M3** (Mixed type), it was considered such increase might be affected by the cream base and not containing astaxanthin (See Attachment III).

##### **4-6-4. Wrinkle-coefficient outer canthi / Skin grain-coefficient of cheeks**

**Fig.8** shows changes of wrinkle-coefficient and skin grain-coefficient after 2 week-application of the cosmetic preparation combined with astaxanthin. Wrinkle-coefficient (deep wrinkles), **S2** of left and right outer canthi, and skin grain-coefficient (Grain uniformity), **K4** of left and right cheeks were measured. The smaller values indicate the smaller or the less in wrinkles and the finer in skin grain-uniformity. Decreases of both wrinkle-coefficient and skin grain-coefficient were observed in **M2** (Dried skin). In this subject, wrinkles of the outer canthi were improved and skin grains of the cheeks were uniform. On the contrary, any changes were not observed in other 2 subjects.

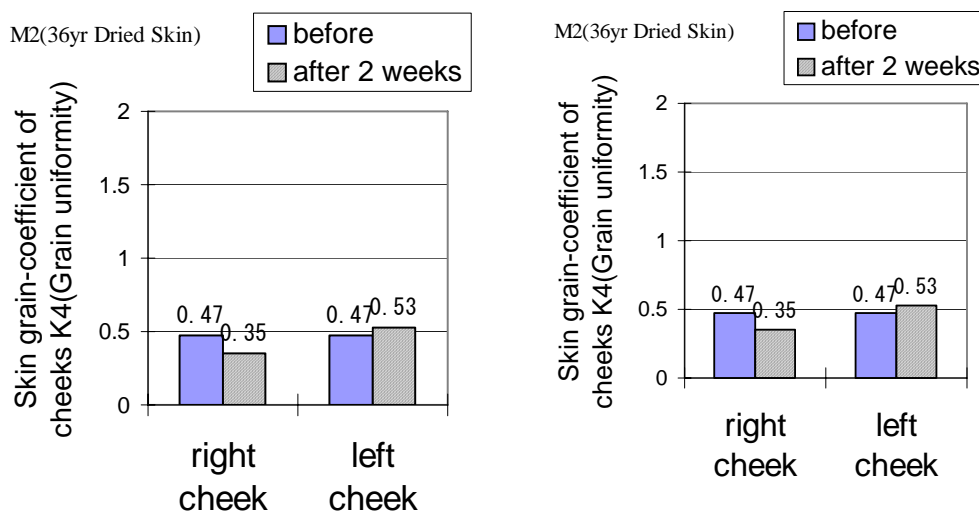


Fig.8. Changes of wrinkle-coefficient outer canthi / skin grain-coefficient of cheeks after 2 week-application of Astaxanthin Combined Cosmetics

#### 4-6-5. Evaluation using magnified photograph of skin

Fig.9 shows magnified photographs of skin in M1 where improvement of fine wrinkled skin condition was observed. Furthermore, mitigation of fine wrinkles under eyes and outer canthi caused by dryness was observed in all 3 cases.

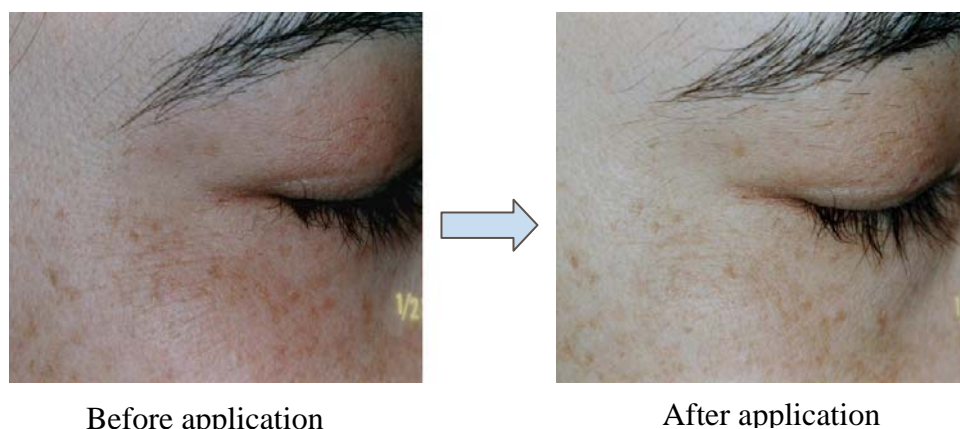


Fig.9. Features of fine wrinkles under eyes and outer canthi (ex: M1)

#### 4-7. Conclusion

After 2 week-application of the astaxanthin cream-preparation, increased skin moisture retention was observed and improvements of fine wrinkles on outer canthi and skin grain uniformity of cheeks were confirmed.

#### 5. Discussion

We studied the effects of astaxanthin from *Haematococcus pluvialis* on

human skin and first confirmed its safety for the skin in Patch Testing and Skin Repeated Application Test and at the same time, the increase of skin moisture retention was observed in the latter study using a cream preparation. Secondly, we then carried out the preliminary study for beauty effect using 3 subjects with normal skin, dried skin or mixed skin type respectively and confirmed the improvement in such parameters as moistness, smoothness and elasticity of the skin obtained by skin-inspection and skin-palpation. Furthermore we confirmed the increase of skin moisture content (skin moisture retention) and the improving tendency of the fine wrinkles outer canthi and skin grain of cheeks.

Since it was clarified in these studies that astaxanthin from *Haematococcus pluvialis* is a promising material combined with cosmetics, we will perform further investigations in order to reconfirm its beauty effect and to elucidate a mechanism of its action.

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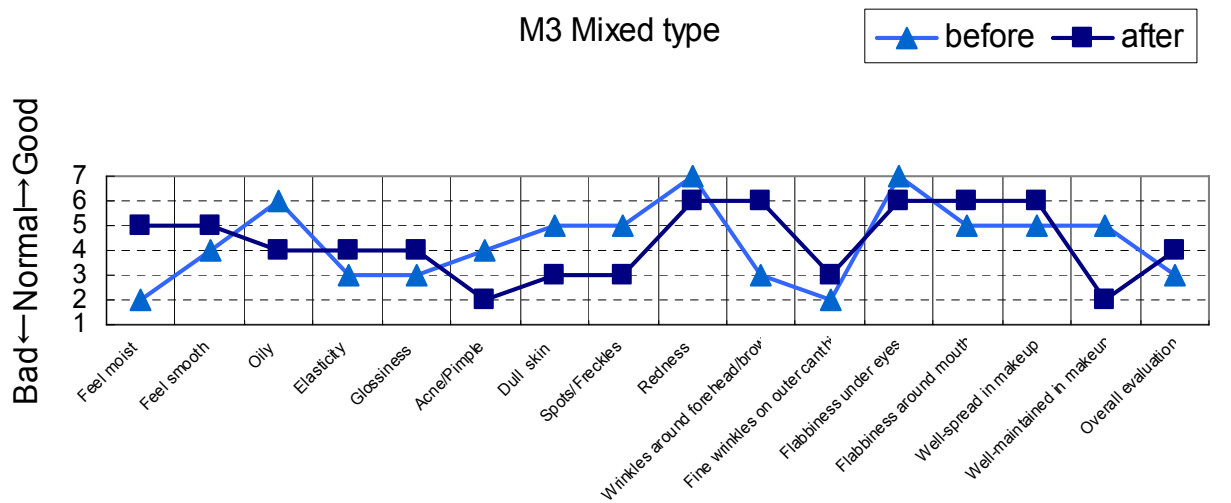
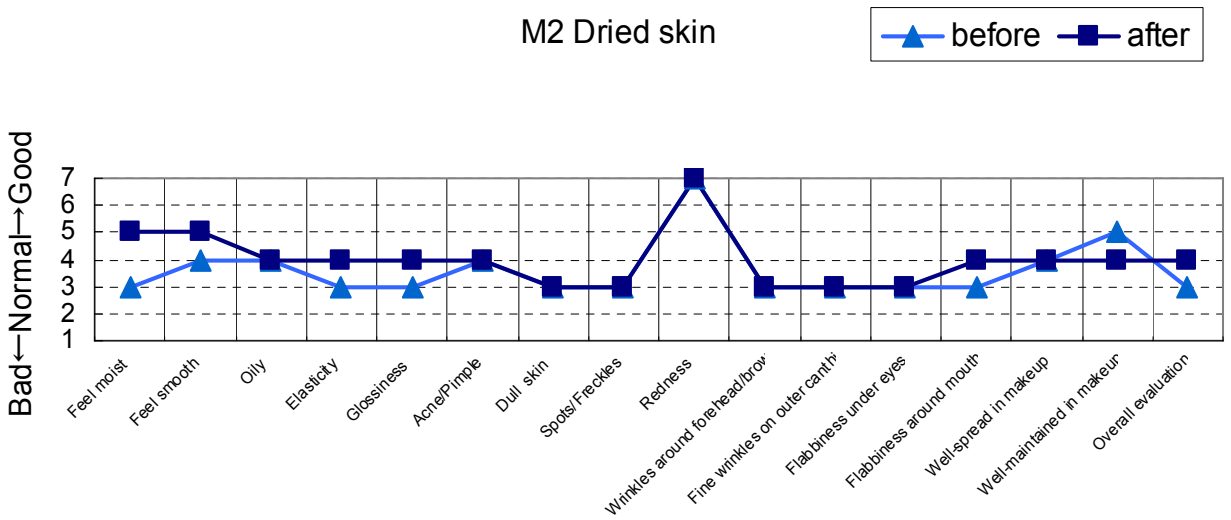
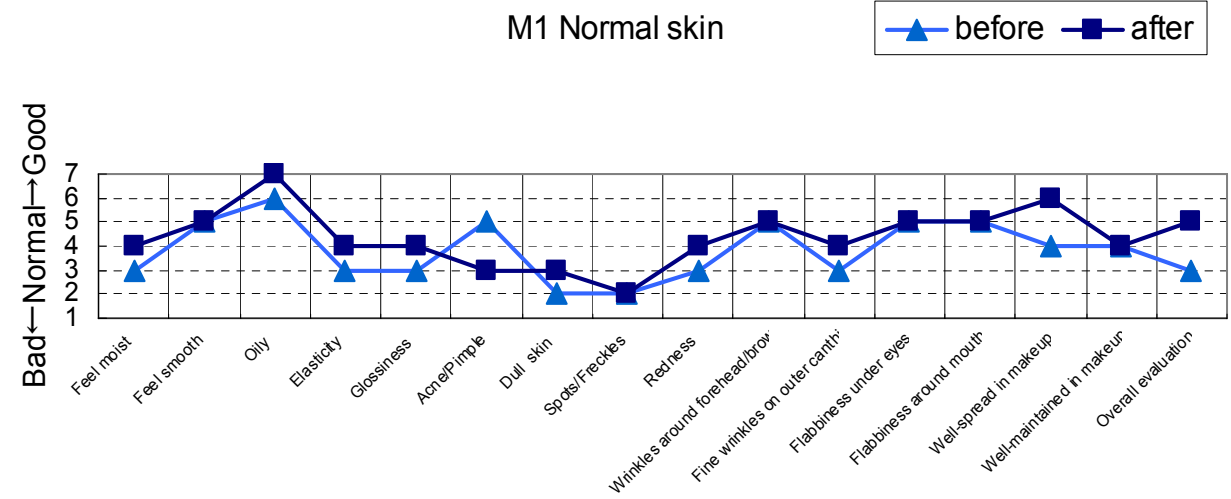


Fig.5 Skin condition in each item evaluated by subject's self-assessment after 2 week-application

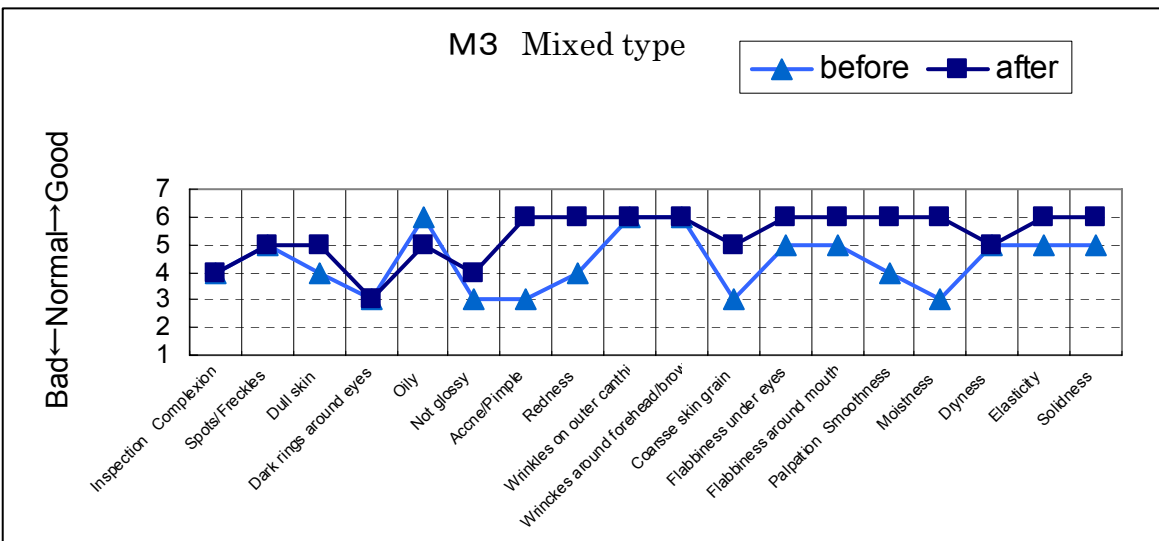
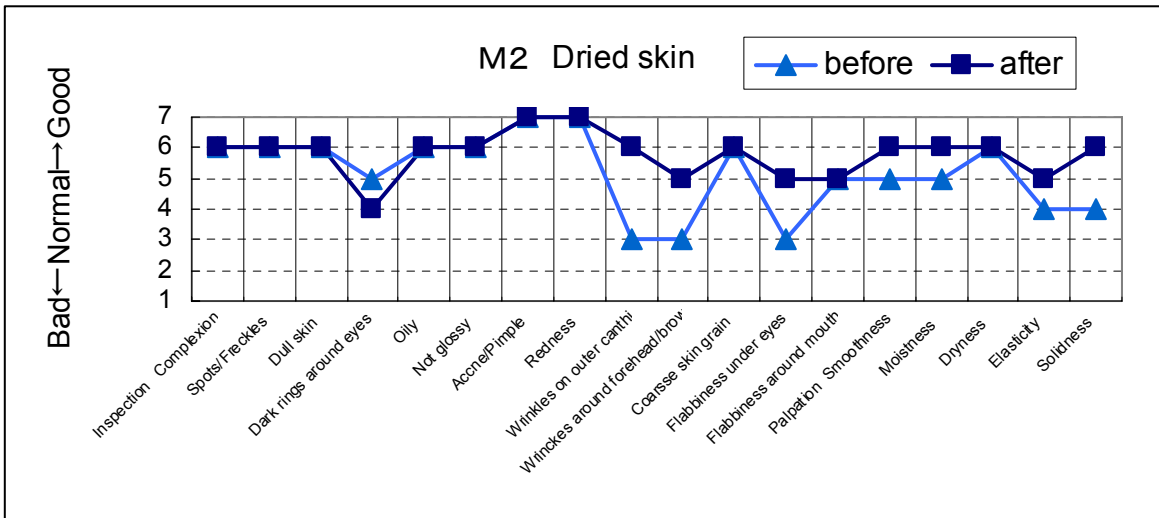
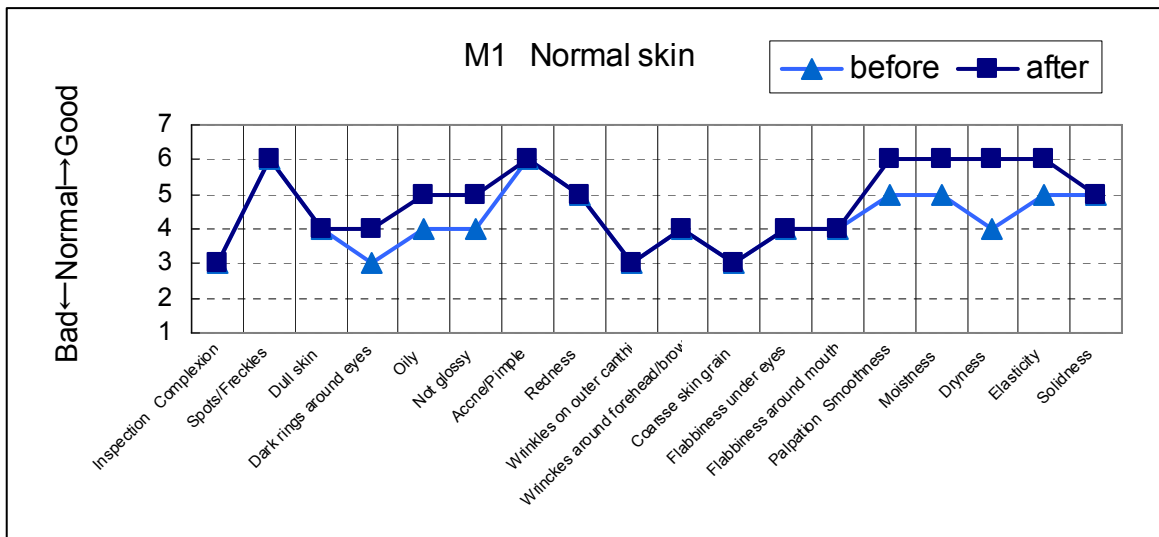


Fig.6. Skin-inspection / skin-palpation by beauty specialist after 2 week-application

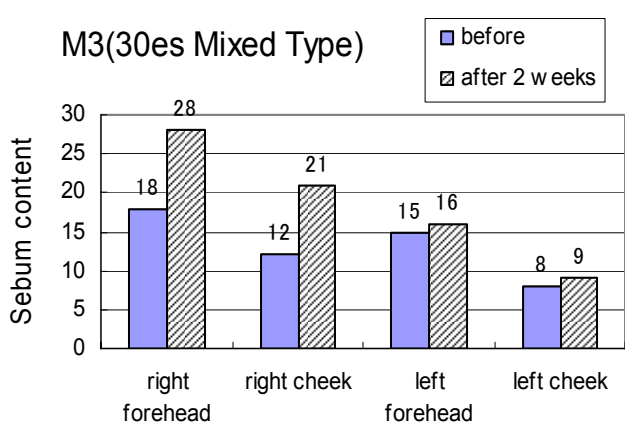
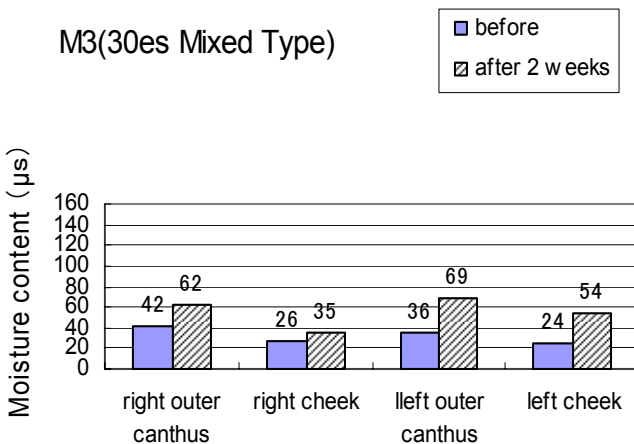
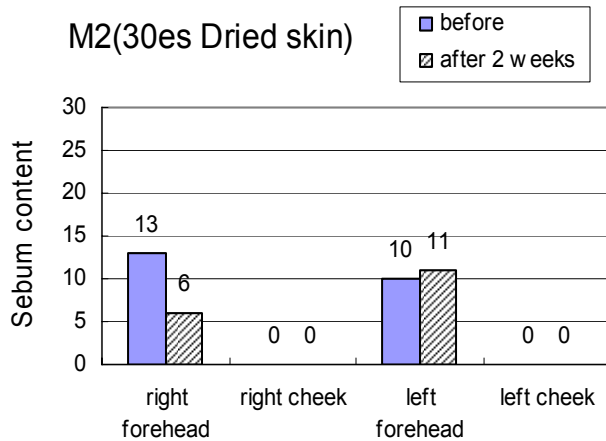
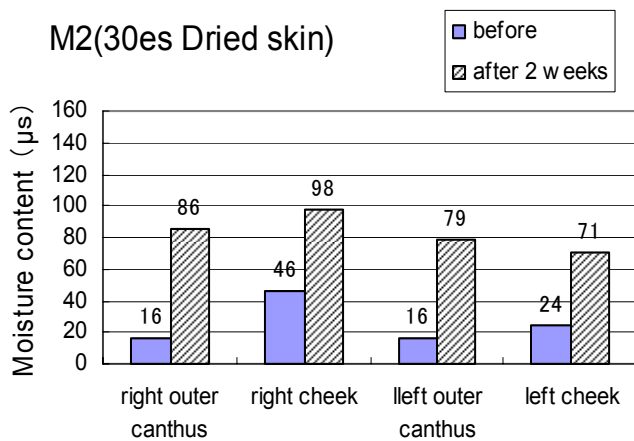
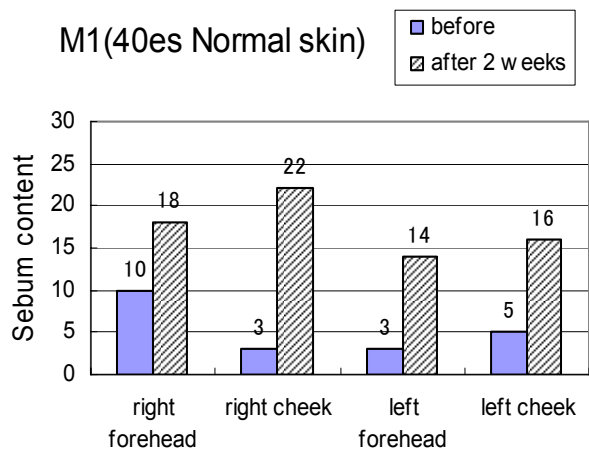
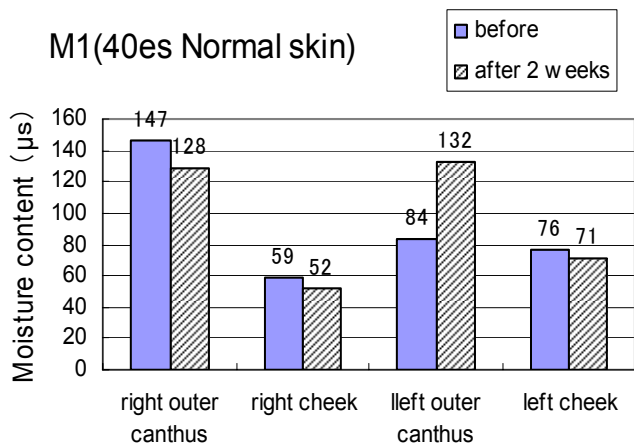


Fig.7. Changes of skin moisture / sebum levels after 2 week-application