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### **"A target approach to textured hair: hair treatment containing murumuru butter for children"**

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#### **1. Introduction**

Hair is a unique character found on all mammals but not on other animals. In humans it is a special and cherished feature, especially in females, but its main functions are in protection of the skin from mechanical insults and to facilitate homeothermy; eyebrows and eyelashes, for example, stop things entering the eyes, while scalp hair prevents sunlight, cold, and physical damage to the head and neck [1].

Humans have several different types of hair that can be classified depending on their body position and form. Moreover, size, angle of penetrance through the skin, embryological time of first appearance, and structural variations in the hair follicles (hair follicle density, size of follicular orifices, hair shaft diameter, volume, and surface of the infundibula) are all taken into account when classifying hair types [1]. Human hair could be classified as (i) androgen-independent hair (i.e., eyebrows and lashes); and (ii) hair on hormone-dependent body regions (i.e., scalp, beard, chest, axilla, and pubic region), which consist of terminal hair shafts, which are long (>2 cm), thick (>60 mm in diameter), pigmented, and medullated [1].

Criteria for classification of hair have included gross size, time of appearance during the life span, and structural variations. Lanugo, or primary hair, is characteristic of the fetal stage of life. It tends to be fine and silky, is nonmedullated and may be considerably pigmented. Secondary, or vellus, hair is short, fine, and usually unpigmented; the downy underfur of some mammals and the fine body hair of children and women are probably characteristic. Some authors restrict the term vellus to such fibers rather than to any type of scalp hair. The tertiary, or terminal, hair is normally considered to be long, coarse, and pigmented, and associated with the mature individual [2].

Vellus hair continues to grow throughout life even in the areas usually considered to have only terminal hairs, such as the scalp, where vellus hair may constitute 6% to 25% of the hair population [3].

Children's hair is on the average finer, rounder, less frequently medullated, and lighter in color than adults' hair [2]. The light color of children's hair is probably related to the low density of pigment, narrow shaft diameter, poorly developed medulla, and largely unpigmented cuticle. Hair form alters under the influence of hormones. Changes from straight to curly and vice versa may occur in scalp hair at puberty [3].

In general, studies on ethnic-racial relations in childhood mention the dimension of hair given that it is an important element in aesthetic appearance, especially for women and girls [4,5,6]. Qualitative study about hair care experiences of African American female adolescents explored the psychosocial importance of hair during childhood and concluded that hair is linked to self-esteem and cultural identity, underscoring the need for proper hair care practices that respect both individual and cultural preferences [7].

Once children's hair differs from adult hair in terms of structure and physiological characteristics, proper care during early development is essential to maintain scalp health and ensure the longevity and quality of hair fibers, and also respect the hair care needs and cultural preferences of this age group.

Despite the growing consumer demand for children-specific hair care products, much of the current market relies on adaptations of adult formulations rather than products developed specifically with pediatric needs in mind. To meet this growing market demand, this study focused on the development and the objective effectiveness evaluation of a specific hair care treatment for children with textured hair containing murumuru butter.

## **2. Materials and Methods**

The hair care treatment for children with textured hair was composed of rinse-off and leave-in formulations: shampoo, conditioner and combing cream (leave-in). They were developed using the combination of relevant ingredients such as: surfactants, co-surfactants, polymers, preservatives, silicone free emollients and Murumuru butter (*Astrocaryum murumuru*).

### **2.1 Investigation on Substantivity of Human Hair via Scanning Fluorescence Microscopy**

The purpose of this study was to assess the level of internal and surface substantivity of the product on human hair fibers via fluorescence microscopy analyses.

The tresses were prepared from curly children's hair, weighing 5.0 g each and 25 cm long. All tresses underwent a standard pre-cleaning process with 10% Sodium lauryl ether sulfate (SLES) solution for 1 minute then rinsed with running water.

Tresses treatment:

\_Control Group (CTRL): Wet the tress for 20s and remove excess water. Apply 1.0 mL of SLES 10% and rub the tress for 60 seconds. Rinse the tress for 30 seconds and remove excess water.

\_Treatment group: Wet the tress for 20s and remove excess water. Apply 1.0 mL of Shampoo and rub for 60 seconds. Rinse the tress for 30 seconds and remove excess water. Apply 1.0ml of the Conditioner on the tress. Rub the tress for 60 seconds. Leave it on the tress for 2 minutes. Rinse the tress for 30 seconds, removing the excess water. Apply 0.5 mL of the Leave-in rub the tresses for 60 seconds. Scrunch the hair tress 3 times. Do not rinse.

After the application of the products, the tresses were dried in a standardized environment at  $55 \pm 5\%$  relative humidity and  $22 \pm 2^\circ\text{C}$ , during 24 hours. Then, the hair fibers were collected, randomly from the tresses. The analysis was performed by using the technique of fluorescence microscopy.

For Surface fluorescence: The hair fibers were collected randomly. Then the hair fibers were embedded in a solution of Rhodamine B ( $8 \text{ g.mL}^{-1}$ ) for 2 minutes followed by rinsing with deionized water for 1 minute and drying. The analysis by fluorescence microscopy was performed for the hair fibers longitudinally arranged in a glass slide for microscopy using the Olympus BX53 microscope with the U-FGW filters. From the images, fluorescence intensities of 30 hair fibers were measured per treatment.

For Cross Sectional Fluorescence: The hair fibers were collected randomly after 24 hours of product application. After the hair fibers were immersed in a Rhodamine B ( $8 \text{ g.mL}^{-1}$ ) solution and drying. Then the hair fibers were embedded in acrylic resin – Technovit® 7100, according to the resin drying and curing procedures. After the hardening of the resin, 10 thick cross sections were made using a glass knife and an ultramichrotome (Reichert-Jung, Heidelberg, Germany). The cross section hair fibers were analyzed using the Olympus BX53 Fluorescence Microscope, with the U-FGW filters. From the images, fluorescence intensities of 30 hair fibers were measured per treatment.

The “Reduction of Fluorescence Intensity, RI”, in relation to the CTRL group treatment, in percentage and number of times was calculated as shown below:

$$RI\% = 100 * ((I\%_{CTRL} - I\%_{TRT}) / I\%_{CTRL})$$

$$RI = I\%_{CTRL} / I\%_{TRT}$$

Where: RI% = Reduction of Fluorescence Intensity in percentage; RI = Reduction of Fluorescence Intensity in number of times; I = Fluorescence Intensity;  $I\%_{CTRL}$  = Values of Fluorescence intensity of the Control Group;  $I\%_{TRT}$  = Values of Fluorescence intensity of the Treatment Group.

## 2.2 Hair Breakage Reduction

The purpose of the present study was to assess hair breakage after the hair care treatment application. The tresses were prepared from child curly 3C hair, weighing 2.5 g each and 25 cm long each. All tresses underwent a standard pre-cleaning process with 10% Sodium lauryl ether sulfate (SLES) solution for 1 minute then rinsed with running water.

Tresses treatment:

\_Control Group (CTRL): Wet the tress for 20s and remove excess water. Apply 0.5 mL of SLES 10% and rub the tresses for 60 seconds. Rinse for 30 seconds and remove excess water.

\_Treatment group: Wet the tress for 20s and remove excess water. Apply 0.5mL of Shampoo and rub the tress for 60 seconds. Rinse the tress for 60 seconds removing excess water. Apply 0.5mL of the Conditioner and rub the tresses for 60 seconds. Rinse for 30 seconds and remove excess water. Apply 0.25mL of the leave-in in the dry hair and rub the tresses for 60 seconds. Do not rinse.

Successive Automated Combability Tests: After the treatment, the tresses were manually combed 5 times to disentangle and then placed on the Automated Hair Comber. The equipment temperature was set at 80°C and rotating speed was set at 25rpm. Tresses were combed for 10 minutes totalling 1000 combing operations. This sequence is one cycle. The procedure was repeated until complete 5 cycles. The tresses were removed from the equipment and then broken hair fibres were counted.

The “Reduction of Broken Fibers, RF”, in relation to the CTRL group treatment, in percentage and number of times was calculated as shown below:

$$RF\% = 100 * ((F_{CTRL} - F_{TRT}) / F_{CTRL})$$

$$RF = F_{CTRL} / F_{TRT}$$

Where: RF% = Reduction of Broken Fibers in percentage; RF = Reduction of Broken Fibers in number of times;  $F_{CTRL}$  = Number of Broken Fibers of the Control Group;  $F_{TRT}$  = Number of Broken Fibers of the Treatment Group.

### **2.3 Study of macro-visual effects on hair - Control of the Frizz effect**

The purpose of this study was to assess reduction in the frizz effect of hair tresses submitted to the hair care treatment by means of image analysis.

The tresses were prepared from curly children's hair, weighing 5.0 g each and 25 cm long. All tresses underwent a standard pre-cleaning process with 10% Sodium lauryl ether sulfate (SLES) solution for 1 minute then rinsed with running water. Tresses were dried in a standardized environment at  $55 \pm 5\%$  relative humidity and  $22 \pm 2^{\circ}\text{C}$ , during 24 hours before tests.

Tresses treatment:

\_Control Group (CTRL): Wet the tress for 20s and remove excess water. Apply 1.0 mL of SLES 10% on the tress. Rub the tress for 60 seconds. Rinse for 30 seconds and remove excess water.

\_Treatment group: Wet the tress for 20s and remove excess water. Apply 1.0 mL of Shampoo and rub for 60 seconds. Rinse the tress for 30 seconds and remove excess water. Apply 1.0ml of the Conditioner on the tress. Rub the tress for 60 seconds. Leave it on the tress for 2 minutes. Rinse the tress for 30 seconds, removing the excess water. Apply 0.5 mL of the Leave-in rub the tresses for 60 seconds. Scrunch the hair tress 3 times. Do not rinse.

After the application of the products, the tresses were dried for 24 hours in a controlled environment at  $55 \pm 5\%$  relative humidity and  $22 \pm 2^\circ\text{C}$  (Initial condition). Then, the tresses were kept in an environment at  $85 \pm 5\%$  relative humidity and  $22 \pm 2^\circ\text{C}$ , for 24 hours. The tresses were photographed in the initial condition, and after 24 hours in the controlled environment at 85% R.H. During the image taking process, a photograph tabletop is used, where lighting and distance are controlled. The original images were converted to a scale of grey using the software program. The frizz was determined as the percentage in area of black pixels relating to frizzy hair fibres (detached from the body of the tress), obtained from the digital image after binarization (conversion to black/white).

The “Reduction of Frizz, RF”, in relation to the CTRL group treatment, in percentage and number of times, were calculated as shown below:

$$RF = 100 * ((F_f^{TRT} - F_f^{CTRL}) / (F_f^{TRT} - F_f^{CTRL}))$$

Where:  $RF$  = Reduction of Frizz of the treatment compared to the control group;  $F_f^{CTRL}$  = Values of frizz for the Control Group;  $F_f^{TRT}$  = Values of frizz for the Treatment Group; f = final; i = initial.

## **2.4 Evaluation of the tensile properties of hair fibers**

The purpose of this study was to assess the mechanical resistance of the structure of hair submitted to hair care treatment. The tresses from children's kinky hair were prepared, weighing 2.5 g each and 25 cm long. All tresses underwent a standard pre-cleaning process with 10% Sodium lauryl ether sulfate (SLES) solution for 1 minute then rinsed with running water.

### **Tresses treatment:**

\_Control Group (CTRL): Wet the tress for 20s and remove excess water. Apply 0.5mL of SLES 10% and rub the tress for 60 seconds. Rinse the tress for 60 seconds removing excess water.

\_Treatment group: Wet the tress for 20s and remove excess water. Apply 0.5mL of Shampoo and rub the tress for 60 seconds. Rinse the tress for 60 seconds removing excess water. Apply 0.5mL of the Conditioner and rub the tresses for 60 seconds. Rinse for 30

seconds and remove excess water. Apply 0.25mL of the leave-in in the dry hair and rub the tresses for 60 seconds. Do not rinse.

The EMIC instrument, model DL500 equipped with a dynamometer with a 20N load cell was used in this test. The tresses were collected from the 3 tresses of each group. Each strand was held by a lower claw and an upper claw connected to the load cell of a dynamometer on the upper part. The following parameter was assessed: Elongation at break and Force at 20% of elongation. The load cell was pre-charged, and the increase in the load was measured with traction speed rates of 100 mm/min. Means and standard deviations were calculated using the GraphPadTM Prism® 6.0 software program.

The “Variation of Force, VF”, promoted by the treatment in comparison with the Control group, in percentage and number of times, were calculated as shown below:

$$\begin{aligned} \text{VF\%} &= 100 * ((F_{T\text{RT}} - F_{C\text{TRL}})/F_{C\text{TRL}}) \\ \text{VF} &= F_{T\text{RT}}/F_{C\text{TRL}} \end{aligned}$$

Where: VF% = Variation of Force in percentage; VF= Variation of Force in number of times;  $F_{C\text{TRL}}$  = Force of the Control Group;  $F_{T\text{RT}}$ = Force of the Treatment Group.

## 2.5 Assessment of the Curl Definition Effect

The purpose of this study was to evaluate the attribute definition curls by means of digital image analysis after application of products for hair care treatment.

In this study, tresses of naturally curly caucasian hair were submitted to the treatments with the products: shampoo, conditioner and leave-in, weighing 5.0 g each and 25 cm long. All tresses underwent a standard pre-cleaning process with 10% Sodium lauryl ether sulfate (SLES) solution for 1 minute then rinsed with running water. Tresses were dried in a standardized environment at  $55 \pm 5\%$  relative humidity and  $22 \pm 2^\circ\text{C}$ , during 24 hours before tests. Then, the tresses were submitted to the treatment.

### Tresses treatment:

\_Control Group (CTRL): Wet the tress for 20s and remove excess water. Apply 1.0ml of the SLES 10% on the tress. Rub the tress for 60 seconds. Rinse the tress for 30 seconds and remove excess water.

\_Treatment group: Wet the tress for 20s and remove excess water. Apply 1.0 mL of Shampoo and rub for 60 seconds. Rinse the tress for 30 seconds and remove excess water. Apply 1,0ml of the Conditioner on the tress. Rub the tress for 60 seconds. Leave it on the tress for 2 minutes. Rinse the tress for 30 seconds, removing the excess water. Apply 0,5ml of the Leave-in on the tress. Rub the tress for 60 seconds. Scrunch the hair tress 3 times. Do not rinse.

After the application of the products, the tresses were dried for 24 hours in a controlled environment at  $55 \pm 5\%$  relative humidity and  $22 \pm 2^\circ\text{C}$  (Initial condition). Then, the tresses

were kept in an environment at  $85 \pm 5\%$  relative humidity and  $22 \pm 2^\circ\text{C}$ , for 24 hours. The tresses were photographed in the initial condition, and after 24 hours in the controlled environment at 85% R.H. During the image taking process, a photograph tabletop is used, where lighting and distance are controlled.

The parameter Curl Definition, CD, was determined according to the results of Frizz and Volume of the tresses.

$$\text{Curl Definition (\%)} = 100 - \text{Frizz} - \text{Volume}$$

Frizz is determined as the percentage in area of black pixels of the binarized image that corresponds to the frizzy hair (hair fibers detached from the body of the tress). Volume is determined as the percentage in area of black pixels on the body of the tress, obtained from the digital image after binarization.

### 3. Results

#### 3.1 Investigation on Substantivity of Human Hair via Scanning Fluorescence Microscopy

The fluorescence intensity data obtained for the treatment group was statistically compared with the Control group by using the method One-way ANOVA, with multiple comparison pot-test Dunnett, considering a 95% confidence interval. According to the results obtained, the tresses to the treatment showed the value of surface fluorescence intensity significantly lower when compared to the tresses submitted to the control group. The results obtained are demonstrated in Table 1.

Table 1. Fluorescence intensity reduction of the treatment in comparison to the CTRL group.

<b>Treatment</b>	<b>Surface</b>		<b>Cortical</b>	
	<b>%</b>	<b>Number of times</b>	<b>%</b>	<b>Number of times</b>
<b>Treatment group (Shampoo, Conditioner and leave-in)</b>	24	1.3	18	1.2

The higher the intensity of fluorescence, the greater the amount of dye attached to the damaged sites. When a product with high substantivity is applied to hair, there is a link between active ingredients and the damaged sites of the hair and, thus, the number of sites available for binding with the dye marker is decreased. Consequently, the intensity of fluorescence is lower.

#### 3.2 Hair Breakage Reduction

Table 2 illustrates the results obtained by counting the total number of broken hair fibers per tress after 5 cycles of successive combings, using Automated Hair Comber equipment.

Table 2. Total Number of broken fibers after 5 cycles of successive combings. Mean  $\pm$  standard deviation.

Cycle	Control Group	Treatment Group (Shampoo, Conditioner and leave-in)
1	52	43
2	46	46
3	47	41
4	49	45
5	50	45
mean $\pm$ SD	49 $\pm$ 2,4	45 $\pm$ 2

The data obtained for the treatment was statistically compared to the CTRL group using one-way ANOVA, followed by a Dunnett's post-test, considering a 95% confidence interval. According to the results obtained, the tresses submitted to the treatments showed a significantly lower number of broken fibers when compared to the tresses submitted to group CTRL. This result is due to the lower friction during the combing, after the treatment group application.

Table 3 shows the result of "Reduction in Broken Fibers" (in percentage and number of times, respectively) of the treatment in comparison to the CTRL group.

Table 3. Reduction of Broken Fibers (% and number of times) of the treatment in comparison to the CTRL group

Treatment	%	Number of times
Treatment group (Shampoo, Conditioner and leave-in)	10	1.1

### 3.3 Study of macro-visual effects on hair - Control of the Frizz effect

According to the results obtained, the tresses from the control and treatment groups at the Final condition showed significantly ( $P<0.05$ ) higher values of Frizz when compared to the tresses at the Initial condition. The comparison of the group CTRL with the treatment group at the initial and final condition, was performed by using the one-way ANOVA, followed by a Dunnett's post-test, considering a 95% confidence interval.

According to the results obtained, the tresses from the Treatment group showed significantly ( $P<0.05$ ) lower Frizz values when compared to the CTRL group at the Initial and Final (after 24 hours of exposure to the 85% R.H. environment) condition.

Table 4 illustrates the "Reduction of Frizz, RF", in percentage and number of times, calculated for the treatment in comparison to the CTRL group.

Table 4. Reduction of Frizz of treatment group compared to CTRL group in percentage and number of times

Treatment	%	Number of times
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<b>Treatment group</b> (Shampoo, Conditioner and leave-in)	81	1.8
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### 3.4 Evaluation of the tensile properties of hair fibers

According to the results obtained, the tresses submitted to the treatment showed no significant difference on elongation at breakage and higher values of force at 20% of elongation when compared to Control group.

Table 5 summarizes the results obtained from Variation of Force (in percentage and number of times) of treatments in relation to group CTRL.

Table 5. Variation of Force (percentage and number of times).

<b>Treatment</b>	<b>%</b>	<b>Number of times</b>
<b>Treatment group</b> (Shampoo, Conditioner and leave-in)	24	1.2

### 3.5 Assessment of the Curl Definition Effect

The results of Curl definition obtained for the Initial condition were statistically compared to the values obtained after 24 hours of exposure to the  $85 \pm 5\%$  R.H for each group, by using the Student's t-test, bimodal, paired, considering a 95% confidence interval.

According to the results obtained, the tresses from the CTRL and treatment groups at the final condition showed significantly ( $P<0.05$ ) lower values of Curl Definition when compared to the tresses at the initial condition.

The comparison of the CTRL group with the treatment at the initial and final condition, was performed by using the one-way ANOVA, followed by a Dunnett's post-test, considering a 95% confidence interval. According to the results obtained, the tresses submitted to the treatments showed significantly ( $P<0.05$ ) higher values of Curl of Curl Definition at the initial and Final condition when compared to the group CTRL.

**Table 6.** Values of Curl Definition (%)

	<b>Control Group</b>	<b>Hair treatment</b>
initial (%)	72,0	91,50
after 24hours (%)	58,1	86,90

The Variation of the Curl Definition (%) of the treatments compared to the CTRL group was 86%. Thus, the treatment "Shampoo + Condicionador + leave-in showed necessary efficacy to support the following claim: Curl Maintenance and Curl Definition, when compared to shampoo without conditioning agents.

## 4. Discussion

Curly children tresses submitted to treatment presented a significantly lower intensity of surface (24%) and cortical (18%) fluorescence in relation to the control group. The treatment provided 24% more strength to the kinky hair and 10% reduction of broken fiber using curly tresses. Higher values of curl definition (86%) and lower frizz values (81%) were also obtained when compared to the control group. These results indicate that this children's hair care treatment containing murumuru butter is well-suited for this fiber type. The results highlight the treatment ability to provide effective curl definition, frizz reduction and improved strength. Additionally, it was possible to confirm that there is an internal repair and deep nourishment of the hair fiber.

## 5. Conclusion

These outcomes support the potential of murumuru butter as a key ingredient in children's hair care products, reinforcing its role in promoting substantivity, strengthening, curl maintenance and frizz control in combination with other ingredients.

## 6. References

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