Journal of Scientific & Industrial Research
Vol. 74, March 2015, pp. 171-175

Quality assessment of chromatophores isolated from squid skin as natural pigment in formulation of lipstick

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Received 23 November 2012; revised 30 June 2013; accepted 30 January 2015

Lipsticks are made to appeal to the current fashion trend and come in a wide range of colors. Lipstick is the only cosmetic ingested, and because of this strict controls on ingredients, as well as the manufacturing processes, are imposed. As long as cosmetics remain in fashion, the market for lipstick will continue to be strong, adding markets in other countries as well as diversifying currently identified markets. But the pigments used in lipsticks can cause health hazards like infertility, anemia and cancer, as well as learning disabilities, mental retardation and behavioral problems, nausea, attention deficit, headaches, skin irritation, etc. The FDA has laid strict regulations regarding the use of lead as a lipstick ingredient. In the present study the chromatophores from squid skins were isolated, and used as natural pigment in lipsticks. Five shades (SQ1, SQ2, SQ3, SQ4 and SQ5) were developed using this pigment and they were screened for consumer acceptance applying hedonic scale method (0 to 9). Comparison with the instrumental reading was also done for the sensory scores. The newly formulated lipstick (SQ2) was subjected to physical, chemical and microbiological quality evaluation. The product was compared with the commercially available lipstick brand and the new product was found to have superior properties compared to the latter ($p<0.01$). It was also found that the new product met with the national quality standards laid down for such category of products. The new product gives a way to utilize squid skin, which is not otherwise useful.

Keywords: Lipsticks, natural ingredients, pigments, chromatophores, health hazards, cosmetics.

Introduction

In recent years, the rise of worldwide living standard has created a demand for cosmetic products. The consumer demands that cosmetic products be of high quality, safe for daily application (without creating any long or short-term side effects to skin) reasonably priced to be affordable to the working class, contain ingredients that have multiple abilities and long-lasting [1]. Cosmetic formulations vary greatly in texture, colour, and physical and chemical properties. The ingredients and basic material used in cosmetic formulations become the important criteria for customers in choosing the cosmetic product, as their interest in health and safety issues grew. In order to meet these challenges, application of natural cosmetic ingredients plays an important role in modern cosmetic production [2]. Currently, there has been a growing interest in natural ingredients which are readily available from seafood discards. However, lack of adequate utilization technology to fully convert such wastes into value-added products must be seriously addressed. The squid skin discarded from the processing plants has high potential benefits hidden behind it. The aim of the present study is to explore the possibility of utilization of squid skin for the isolation of chromatophore and its application in lipstick as a natural colourant.Consumers and health care providers should be aware that many toxic synthetic chemicals are used in cosmetics and personal care products without full disclosure on package labeling. A study by US consumer group *Campaign for Safe Cosmetics*, in October 2007 found 60 percent of lipsticks tested contained trace amounts of lead, especially in red lipsticks [3]. The levels of lead varied from 0.03 to 0.65 parts per million. Fragrance chemicals have neurological effects that can alter blood pressure, pulse, mood, and have a sedative effect[4]. Chemically sensitive people may get negative symptoms that include headaches, nausea, and lack of concentration, asthma, fatigue and irritability. In this context an attempt has been made to develop a completely organic lipstick using the chromatophores isolated from the squid skin discards as natural ingredient.

The newly formulated lipstick confirm to the requirements of Bureau of Indian Standards IS 9875:1990. A comparative evaluation between

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commercially available lipstick product and the prepared natural lipstick were also done for its physical, chemical, microbiological and sensory aspects. The consumer acceptance was also investigated.

Material and Methods

Preparation of squid waste

Squid, *Loligo duvauceli* (Orbigny, 1848) were procured from Cochin Fisheries Harbour, Cochin, India. They were immediately iced (1:1 ratio) and transported in an insulated container to the laboratory. They were deiced and thoroughly washed to remove slime and dirt with chilled potable water (0-2 ^{0} C). The skin was then carefully peeled off. The peeled skin is homogenized in a laboratory mixer and moisture content is removed by the addition of required amount of anhydrous sodium sulphate.

Extraction of Chromatophores

Different solvent combinations viz. Hexane: Isopropyl Alcohol (2:3, 3:2, 0:5) and Acetone alone were tried for the extraction of chromatophore. Isopropyl alcohol and acetone when used alone, chromatophores could not be extracted out. Chromatophore yield from Hexane: Isopropyl Alcohol combination in 2:3 was only 0.5%. Whereas combination of Hexane: isopropyl Alcohol (3:2) gave highest yield of (2%) chromatophores. This combination was selected for further studies. The solvent extract was washed with saline and the pigmented layer was separated and evaporated under vaccum. The extract was suspended in the same solvent and was passed through a column filled with silica and then through a column filled with carbon. The pooled fractions were further evaporated under vacuum.

Preparation of Lipsticks

Beeswax (15%), Paraffin wax (20%), Vitamin E (4%), Food grade flavor (10%) were used for the preparation of lipstick. Other than these castor oil and colour was also added, both together constitutes 51% of the product. Extracted squid chromatophore in required quantity was used as a natural colourant, instead of other artificial colouring agents.Required amount of beeswax and Paraffin wax were taken and melted in a clean container. Vitamin E and castor oil were added to the melted mixture and continued heating. Then to the mixture required amount of chromatophores was added and mixed thoroughly till no lumps remained. Then flavor was added and mixed well. Poured the melted mixture into the pre-cleaned moulds. Placed the caps and allowed for setting. Lipstick is mixed and processed in a controlled environment so it will be free of contamination. Incoming material is tested to ensure that it meets required specifications. Samples of every batch produced are saved and stored at room temperature for the life of the product (and often beyond that) to maintain a control on the batch.

Quality Control for Lipstick

Physical Analysis

Determination of Melting point: In order to determine the melting point, a sample of 50mg lipstick was taken, melted and filled into a glass capillary tube open at both ends. Cooled the capillary tube with ice for 2 hours, fastened the tube with a thermometer .Placed this assembly in a beaker filled ¾th with water on a heating plate with a magnetic stirrer. Continued heating and stirring at slow and fixed speed. The temperature at which the material moves along the capillary tube is considered as the melting point.

Heat Test: In the Heat test, the Lipstick is placed in the extended position in a holder and left in a constant temperature oven of over 130 degrees Fahrenheit (54^{0}C) for 24 hours. There should be no drooping or distortion of the lipstick.

Colour measurements: Colorimetric analysis were performed with a Hunter lab Miniscan ® XE plus spectrocolorimeter (Hunter Associates Laboratory, Inc. Reston, Virginia, USA).Measurements were recorded using the L* a* b* colour scale (CIE, 1986). The tristimulus colour values (L* lightness, a* redness and b* yellowness) of samples were recorded on CIE scale using spectrocolorimeter, which was earlier calibrated using white and black standard ceramic tiles. The observations were made using D-65 illuminant and 10 degree observer.

Grittiness: Grittiness in lipstick was tested as per IS: 975:1990.

Sensory Analysis

The sensory attributes of the lipsticks were evaluated by a panel of ten trained panelists. The sensory evaluation was carried out by using a 9 point hedonic scale, as described by[5] by giving numerical values of 1 (dislike extremely) to 9 (like extremely). Panelists indicated their rating for each sample by choosing the appropriate numerical score. The sensory attributes assessed included the Hardness,

Consistency, Colour, Stickiness and Overall acceptance .The limit of acceptability was 6.

Microbiological Analysis

The numbers of Total bacterial and fungi colonies per gram of the sample were determined as per IS: 9875:1990.

Chemical Analysis

Rancidity: Rancidity in lipstick was tested as per IS: 9875:1990.

Trace metal Analysis: The sample was digested as per Khemnani *et al*. (2012)[6] with modification. 1gm sample was treated with a mixture of HNO_3 and $HClO_4$ in 9:4 ratios and kept 24 hours at room temperature for pre- digestion. Then the mixture was treated at 50^0 C for 4 hours in a water bath and after that heated over sand bath in low temperature till the end of evolution of white fumes. Filtered the sample using whatman number 40 filter paper and made upto 100ml.This digested lipstick samples were processed for analysis of heavy metals- Magnesium, manganese, nickel, lead, antimony, selenium, cadmium, cobalt, chromium, copper, zinc and iron were analyzed. All these metals were analyzed using Perkin Elmer Optima 2000DV ICP AES.

Statistical Analysis

The data were analysed using one-way analysis of variance and significance between means were tested using Duncan's (1955) multiple range test at $p< 0.01$ levels (SPSS 12.0).

Results and Discussion

The percentage composition of basic ingredients of lipstick is given in the Table 1. To this mix, according to the required shade of chromatophore can be added and a range of colour shades of lipsticks can be made.The tristimulus colour values (L* lightness, a* redness and b* yellowness) of Extracted chromatophores were recorded on CIE scale in triplicate using a Caloriemeter. The Hunter colour scale values for extracted chromatophore was 24.49±1.25, 11.47±0.22 and0.76±0.43 for lightness (L*), redness (a*) and yellowness (b*).According to the different L*, a*, b* colour scale values, the different colour shades can be prepared.

Optimisation of squid chromatophore based Lipstick formulation

Five formulations of Lipsticks with varying colour shades (SQ1, SQ2, SQ3, SQ4 and SQ5) with chromatophore content carried from 1.25%, 1.0%, 0.75%, 0.50% and 0.25% respectively were prepared and the corresponding L*, a*, b* values are shown in Table.1 .On statistical analysis L* values of all shades were found to be significantly different ($p<0.01$) .I n the case of a* and b* values there was no significant difference between SQ2, SQ3 and SQ4. In order to find the consumer preference, a sensory evaluation was also done and the results were depicted in Table.2. Numerical optimisation is performed in order to obtain the formulation with most desired characteristics. The optimization target was based on the instrumental measurement and consumer

Table 1—The L*, a*, b*colour scale values for five shades of lipstick

Colour scale	SHADES				
	SQ 1	SQ 2	SQ 3	SQ 4	SQ 5
L*	26.81±0.54^a	33.98±0.50^b	35.24±0.53^c	37.26±0.53^d	47.98±0.53^e
a*	10.46±0.28^a	10.09±0.38^b	9.22±0.22^b	9.15±0.30^b	7.44±0.82^c
b*	1.05±0.24^a	9.53±0.53^b	10.09±0.44^b	10.22±0.37^b	13.58±0.66^c

All values are Mean± SD of triplicate analysis. Different superscripts in the same raw indicates significant differences ($p<0.01$)

Table 2—Sensory evaluation for five shades of lipstick

parameters	SHADES				
	SQ 1	SQ 2	SQ 3	SQ 4	SQ 5
Hardness	7.8±0.6	8.4±0.5	8.1±0.5	7.9 ±0.5	7.0±0.8
Consistency	8.2±1.2^a	8.3±0.5^b	8.0±1.5^a	7.7±0.8^a	7.5±1.1^a
Colour	7.5±0.5^a	8.3±0.8^b	7.2±0.5^a	7.2±0.4^a	6.2±0.8^a
Stickiness	7.9±0.6^a	8.5±0.5^b	8.0±0.2^a	7.8±0.5^a	6.5±1.2^a
Overall acceptance	8.0±0.5^a	8.7±0.5^b	7.5±0.4^a	7.5±0.8^a	7.2±1.3^a

All values are Mean± SD of triplicate analysis. Different superscripts in the same row indicates significant differences ($p<0.01$)

evaluation data (Table 1). The melting point of formulated Lipsticks was found to be in the range of 59-62^0 C. According to Bryce, 1993, the melting point of lipsticks is generally within the range 55-75^{0}C. According to him the accepted limit for Lipstick melting point was in the range of 60.6 to 64.0 ^{0}C. The melting point of lipstick must be high to avoid it from technical deterioration when expose to anticipated environmental temperature and humidity during preparation and use. Consequently the maximum melting point is desirable for lipstick formulation. On the other hand, for hardness and viscosity of the lipstick, there is no specific requirement. Consumer acceptability in terms of hardness, consistency, stickiness and overall acceptance for the lipstick has been evaluated by using nine point hedonic scale. The acceptability limit for the consumer score is fixed as 6. The highest score indicates the most acceptable formulation. On analyzing the data statistically it was found that, except for the attribute hardness, there was significant difference between SQ 2 and other shades. Out of the five formulations of lipstick shades, the second one (SQ2) was the most preferable one. Hence further studies were carried out with the shade SQ2.

Comparative Evaluation of formulated (SQ2) and commercial Lipstick sample

Table 3 depicts the comparative evaluation of newly formulated lipstick with the commercial lipstick. From the results it was observed that newly formulated lipstick is no way inferior to commercial sample. On statistical analysis (ANOVA) for most of the properties, the prepared sample is superior to the commercial sample, and the significance is shown in the same table. As far as heavy metal analysis is concerned newly formulated lipstick was found to be better than commercial sample. The squid lipstick was having an attractive appearance, pleasant taste and feels on the lips and was reasonably free from sweating, bloom and rancidity, which are the specified requirements for lipsticks as per Bureau of Indian standards (BIS) .In addition the newly formulated product conforms to the requirements of BIS in firmness and texture. The consumer preference study of the formulated lipstick has shown good organoleptic scores. The results of the study are exposed in Table3. The same table also depicts that the product was free from mold growth and was not rancid. The presence of heavy metals in cosmetics can cause serious problems to consumer as they can cause premature aging of the skin, skin allergies, and skin cancer. Further, toxic metals have a role to set up conditions that lead to inflammation in arteries and tissues, results in osteoporosis (Farr, 2009)[7].The analysis of heavy metal content in chromatophore based lipstick sample has shown an acceptable range of element concentration. Magnesium, manganese, nickel, lead, antimony, selenium, cadmium, cobalt, chromium, copper, zinc and iron were analyzed using Perkin Elmer Optima 2000DV ICP AES. Of these,

Table 3—Comparative Evaluation of chromatophore based lipstick (SQ2) with commercial Lipstick

Properties	Prepared sample	Commercial sample
	Physical	
Melting point	59.29±4.27 ^{a}	62.55±5.27 ^{a}
Colour scale L*	33.98±0.50^a	24.52±0.54^b
a*	10.09±0.38^a	10.54±0.58^a
b*	9.53±0.53^a	2.06±0.42^b
Grittiness (free from hard & sharp edged particles)	Nil	Nil
Sensory(Consumer evaluation) maximum score 9		
Hardness	8.4±0.5 ^{a}	8.9±0.5 ^{a}
Consistency	8.6±0.5 ^{a}	8.0±0.5 ^{b}
Colour	8.3±0.8 ^{a}	7.3±0.5 ^{b}
Stickiness	8.5±0.5 ^{a}	8.9±1.2 ^{a}
Overall acceptance	8.7±0.5 ^{a}	8.4±0.5 ^{a}
	Chemical	
Heavy metal (ppm) profiling		
Magnesium	61.72 ^{a} ±1.25	112 ±0.30 ^{b}
Manganese	1.19 ±0.04 ^{a}	1.21 ±0.04 ^{a}
Nickel	9.89 ±0.74 ^{a}	14.6 ±6.3 ^{a}
Lead	3.23 ±0.09 ^{a}	105.5 ±67.5 ^{b}
Antimony	0.35 ±0.53 ^{a}	0.39 ±0.05 ^{a}
Selenium	ND	ND
Cadmium	ND	1.1±0.6
Arsenic	ND	2.013±0.006
Cobalt	ND	ND
Chromium	7.56±1.15^a	31.8±11.2^b
Copper	ND	4.33±0.22
Iron	49.42 ±6.12 ^{b}	297.5 ±167.6 ^{b}
Zinc	9.17 ±2.15^a	94.9 ±38.5 ^{b}
Peroxide value	0.4 meq O2/gm ^{a}	1.2 meq O2/gm ^{b}
	Microbiological	
TBC	26cfu/g ^{a}	38cfu/g ^{b}
Total mould growth	Nil	Nil

All values are Mean± SD of triplicate analysis. Different superscripts in the same row indicates significant differences ($p<0.01$)

selenium, cadmium, cobalt, Arsenic and Copper were not detected. Mn, Ni, Pb, Sb,Cr and Zn were in very negligible level and not harmful to consumers. Magnesium which plays an important role in the maintenance of the skin nutritional requirements and Fe as an essential nutrient necessary for oxygen metabolism and mitochondrial function is present in formulated lipstick in 61.72 and 49.42ppm respectively. According to Landsdown (2001)[8] iron exhibits a functional importance as a trace metal in the normal growth and functional maturation of the skin. Ajasa, 2004[9] and Sharma *et al*, 2011[10] have reported the importance of Mg, Zn and Cu in the structural maintenance of the skin . Cadmium (Cd) is prohibited in any amount in cosmetics (Council Directive 76/768/EEC of 27 July 1976).Toxic heavy metals Cadmium and Arsenic were detected in the commercial lipstick, But these two toxic metals were not detected in the prepared lipstick samples. Indian standards (IS9875:1990) has given a limit for lead as 20ppm. The lead (Pb) content in formulated lipstick (3.23ppm) was significantly ($p<0.01$) lower compared to the commercial sample (105.5 ppm), indicating the superior quality of squid lipstick. Mercury, whose limit prescribed by ACSB (2007)[11] is 1ppm for all cosmetic products could not detect in either of the samples.

Conclusion

The newly formulated chromatophore based lipstick confirm to the requirements of Bureau of Indian Standards IS 9875:1990. After formulation of lipstick physical, chemical, microbiological and organoleptic properties were studied. The consumer's acceptance was also investigated to determine the best formulation of Lipstick. A comparative evaluation between commercially available lipstick product and the prepared natural lipstick were also done for its physical, chemical, microbiological and sensory aspects. The continuous use of these commercial cosmetics could result in an increase in the trace metal levels in human body beyond acceptable limits. The chromatophore based product was free from toxic metals and hence can be considered safe to use. There is an urgent need for constant quality assessment of cosmetic products in the market in order to ensure the safety of consumers. The regulatory bodies and the government should implement stringent policies to regulate and monitor the standards of cosmetic products manufactured, advertized, sold, and used.

Acknowledgements

The authors are grateful to the director, Central Institute of Fisheries Technology, Cochin for the permission to publish the paper.

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