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Influence of nanoencapsulation on the sensory properties of cosmetic formulations containing lipoic acid

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Synopsis

Sensory analysis has become a valuable tool in qualifying consumer perception regarding cosmetic products. This study aims to explore the application of discriminative and affective consumers sensory analysis in evaluating the influence of nanoencapsulation on the sensory properties and rheological characteristics of a cosmetic formulation containing lipoic acid. The nanocapsules were prepared by the pre-formed polymer precipitation method. Semisolid formulations were prepared using a silicone emulsion system, and these pseudoplastic fluids were characterized using rheological methods. The panellists $(n = 88)$ analysed the formulations with and without nanoencapsulated lipoic acid as paired comparisons within the discriminative and affective sensory analysis. In these measurements, spreadability, stickiness, oiliness and sulphur odour were evaluated. The panellists had no previous training in conducting these measurements. It was shown that nanotechnology can change some sensory characteristics of the formulations. The spreadability, stickiness and oiliness are the attributes for which the panellists noted differences with statistical significance. The spreadability difference could be due to the results found in the rheological profiles and consistency indexes between formulations. In the discriminative analysis, the panellists could not detect any noticeable differences in the sulphur odour or residual properties between samples, attributes that would influence whether consumers adhered to the selected treatment. Considering affective analysis, the consumers communicated that the formulation containing the nanoencapsulated lipoic acid, which presented less consistency, was preferred based on the reduction in immediate stickiness and residual sulphur odour. The free lipoic acid formulation was preferred in terms of residual oiliness and spreadability.

Résumé

L'analyse sensorielle est devenue un outil précieux pour évaluer la perception des produits cosmétiques par les consommateurs. Cet article vise à étudier l'application de l'analyse sensorielle discriminatoire et affective des consommateurs dans l'évaluation de l'influence de la nanoencapsulation sur les propriétés sensorielles et les

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caractéristiques rhéologiques d'une formulation cosmétique contenant de l'acide lipoïque. Les nanocapsules ont été préparés par le procédé de précipitation de polymère pré-formé. Des formulations semi-solides ont été préparées l'aide d'un système d'émulsion de silicone et ces fluides pseudo-plastiques ont été caractérisés en utilisant des procédés rhéologiques. Les panélistes ($n = 88$) ont analysé les formulations avec et sans acide lipoïque nanoencapsulé en tant que comparaison par paires a` l'aide de l'analyse sensorielle affective et discriminatoire. Dans ces mesures, l'étalement, la viscosité, l'onctuosité et une odeur de soufre ont été évalués. Les panélistes n'ont eu aucune formation préalable en la réalisation de ces mesures. Il a été montré que les nanotechnologies peuvent changer certaines caractéristiques sensorielles des formulations. L'étalement, la viscosité et l'onctuosité sont les attributs pour lesquels les panélistes ont noté des différences avec des statistiques significatives. La différence de l'étalement pourrait être due aux résultats présentés dans les profils rhéologiques et les indices de consistance entre les formulations. Dans l'analyse discriminante, les panelistes ne pouvaient pas détecter d'éventuelles différences notables dans l'odeur du soufre ou des propriétés résiduelles entre les échantillons, des attributs qui pourraient influencer les choix des consommateurs. Considérant l'analyse affective, les consommateurs ont communiqué que la formulation contenant l'acide lipoïque nanoencapsulé, qui a présenté moins de consistance, a été préférée basée sur la réduction immédiate d'adhérence et de l'odeur résiduelle de soufre. La formulation à l'acide lipoïque libre a été préférée en termes de toucher huileux résiduel et d'étalement.

Introduction

Sensory analysis is a multidisciplinary science that covers the measurement, interpretation and understanding of the human responses to product properties detected through the senses. It has been demonstrated to be of considerable importance in the development of cosmetic products designed to delight the consumer's senses [1, 2]. Sensory properties are the first contact that consumers have with cosmetic products, and they relate such properties to the product efficacy [3].

Sensory analysis is widely used in the product development process, in quality control and in product marketing [2, 4–7]. Using this technique, it is possible to comprehend the consumer preferences in relation to certain products and to modify the formulations and improve them, in order to enhance the product's acceptability by the

target [8, 9]. Sensory analysis allows information to be obtained which is not detected through other analytical procedures, for example, the consumer acceptability and purchase intention. The importance of sensory analysis is well recognized because the cosmetic sensory proprieties directly impact the treatment adherence. Thus, using sensory analysis techniques is a powerful research tool to help better develop innovative cosmetic products [2, 5, 6, 10, 11].

In the last decade, the use of nanotechnology has been proposed for several cosmetic products [12, 13]. However, until now, there is no data available in the scientific literature regarding the impact of nanoparticles on the sensory properties experienced by the consumers using these cosmetic products. Progress in the nanotechnology field enables the development of nanoparticles, which can serve as reservoirs for lipophilic substances[14, 15],prolonging and controlling the percutaneous release of the active substances, besides increasing the adhesivity and the residence time of such substances on the skin [14–19]. Nanoparticle systems present a large superficial area which can lead to higher dermal penetration due to an increase in the contact surface with the stratum corneum. Consequently, the nanoparticles can efficiently bind to the cutaneous surface and increase the coverage area in relation to the non-encapsulated substance, adhering to the skin, forming a protector film and prolonging the cutaneous retention time of lipophilic substances [16–18, 20].

The present application of nanoparticles in cosmetic formulations leverage their ability to increase the stability and the cutaneous tolerance of several raw materials [12]. Several cosmetics containing nanoparticles have been developed with claims of higher efficacy and better sensory properties. To claim, in this context, means to affirm, declare or sustain any information given to the public, primarily with the aim of publicity, regarding the nature, effect, properties or efficacy of a cosmetic product. A claim can be comparative, when related to the properties of other products or non-comparative, when it declares the properties of a product independently [21, 22]. All such claims should be substantiated by consistent justification and clear demonstrations. They should be based on scientific evidence, evaluated by consumers or laboratory

Table I Summary of most common designs used in sensory analysis

studies, including sensory analysis by humans, instrumental tests or biochemical methods. Sensory analysis thus represents a reliable alternative to support claims, if conducted according to an adequate protocol[10, 11, 21–23].

There are different types of tests used in sensory analysis, such as discriminative, affective or descriptive [24, 25], and the objective of the analysis defines the choice of method. For instance, in cases of determining whether there is a difference between samples, the discriminative tests are more suitable. On the other hand, to ascertain the difference between samples or the dimension of that difference, descriptive tests, which use scales and profiles, are the most appropriate. Also, if the main objective is to determine a favoured product or the reason why that product is favoured, acceptance methods are the most appropriate [2, 25]. Table I summarizes the characteristics of the most common designs used in sensory analysis.

In this context, the objective of this study was to explore the application of discriminative and affective consumers sensory analysis in evaluating the influence of nanoencapsulation on the sensory properties and rheological characteristics of a cosmetic formulation containing lipoic acid. Two formulations were compared: one based on the incorporation of the active substance in nanocapsules and the other containing the free substance. The active substance used was lipoic acid, a prominent antioxidant in dermocosmetics, used in the prevention and treatment of skin ageing [26–28]. The formulation was chosen due to the increase in the physicochemical stability of lipoic acid after its nanoencapsulation, protecting the substance from degradation, which leads to an unpleasant specific odour and can decrease the treatment adherence [29–31].

Materials and methods

Materials

Lipoic acid and diazolidinyl urea were of pharmaceutical grade and provided by PharmaNostra (Brazil) and Sarfam (Brazil), respectively, whereas butyl-hydroxy-toluene and Unistab S69® were both

from Via Farma (Brazil). Sorbitan monostearate and poly(ξ-caprolactone) ($Mn = 42$ 500, $Mw = 65$ 000) were supplied by Sigma-Aldrich (France) and silicone DC RM2051 was from Dow Corning (U.S.A.). A mixture of capric and caprylic triglycerides (Ritamollient CCT^*) was purchased from Brasquim (Brazil), polysorbate 80 obtained from Synth (Brazil) and acetone of analytical grade from Nuclear (Brazil). Acetonitrile of chromatographic grade was obtained from Merck (Brazil).

Preparation of nanocapsules and semisolid formulations containing free or nanoencapsulated lipoic acid

The lipoic acid-loaded nanocapsules suspension were prepared by the pre-formed polymer precipitation method[32]. The lipoic acid was weighed (25 mg) and dissolved in the organic phase composed of capric/caprylic triglycerides (0.33 mL), sorbitan monostearate (76.6 mg), poly(ξ -caprolactone) (100 mg), acetone (26.7 mL) and butyl-hydroxy-toluene (BHT) (0.01 g). The organic phase was injected into an aqueous phase containing polysorbate 80 (76.6 mg), diazolidinyl urea (0.01 g) and Milli- $Q^{\text{®}}$ water (53.3 mL), through a funnel and maintained under moderate magnetic stirring for 10 min. The suspension was prepared (in triplicate of batches) protected from light, and the solvents were evaporated in a rotary evaporator (Büchi R-114) at a temperature of approximately 30° C until a final volume of 10 mL. to give a final concentration of 2.5 mg.mL^{-1} .

To obtain the semisolid formulation containing nanoencapsulated lipoic acid (2.5 $mg.mL^{-1}$), the nanocapsules suspensions were thickened with the emulsifying Silicone DC RM2051® (4g) and Unistab S69® (0,5g). The formulation was called N and prepared in triplicate of batches.

Semisolid formulations for cutaneous use of lipoic acid, containing the active compound in its free form, in the same concentration, were obtained by lipoic acid solubilization (25 mg) in capric/ caprylic triglycerides (0.33 mL), the substance which forms the oily core of the nanocapsules. BHT (0.01 g), diazolidinyl urea (0.01 g), Silicone DC RM2051[®] (4 g), Unistab $S69^{\circledast}$ (0.5 g) and Milli- Q^{\circledR} water (volume completed until 10 mL) were then also added. This formulation was called L and also prepared in triplicate of batches.

Physicochemical characterization of the formulations

The nanocapsules suspensions containing lipoic acid were evaluated as previously described by KÜLKAMP and co-workers[30]. The determination of the particle's average diameter and size distribution were performed by dynamic light scattering (Zetasizer Nanoseries, Malvern), after dilution (500 fold) with MilliQ[®] water. The laser wavelength was 532 nm and the scattering angle 173°. The zeta potential was measured by electrophoretic mobility, using the same equipment, after dilution (500 fold) with NaCl 10 mM solution.

The lipoic acid content in suspensions and semisolid formulations was assayed by HPLC method. A Perkin Elmer HPLC system with S-200 auto-injector, and column Waters Nova Pak C18, 4 μ m (3.9 x 150 mm) were used. The mobile phase consisted of acetonitrile: $0.01M$ phosphoric acid (60 : 40, v/v) at a flow rate of 1.0 mL min⁻¹. The method was validated for lipoic acid amount within the range from 30 to 120 μ g. mL⁻¹ dilution, in terms of linearity, precision, accuracy, detection and quantification limits and specificity. The total lipoic acid content in suspensions and semisolid formulations was determined after extraction with acetonitrile from the suspensions or semisolid formulations and filtration (0.45 $\mu \textrm{m}$, Millipore®), at an expected concentration of 50 μ g mL⁻¹ after this preparation procedure.

The lipoic acid encapsulation efficiency was determined after separation of the free lipoic acid from the nanostructures in the suspensions by ultrafiltration-centrifugation using Microcon 10 000 MWCO (Millipore) membranes. The centrifugation was performed at $2200 \times g$ for 10 min (Microcentrifuge Sigma1-14), and lipoic acid determined by the same HPLC method described above. The amount of lipoic acid associated with the nanostructures was determined by the difference between the total amount of lipoic acid in the formulation and free lipoic acid determined after the ultrafiltration-centrifugation method. The semi-solid formulations were also characterized regarding their pH, without previous dilution (potentiometry, Denver instruments, Bohemia, NY, U.S.A.).

Rheological characterization of the formulations

The rheological characterization was performed using a Brookfield viscometer (Brookfield DV-II + Pro, LVF mode, spindle SC4-25) with a shear rate interval of 0.44 to 2.42 rpm. The samples (16 mL) were maintained in a circulating water bath under controlled temperature (26 \pm 2 °C). The data were analysed using the Bingham eqn (1), Casson eqn (2), Ostwald eqn (3) and Herschel– Bulkley eqn (4) flow models.

$$
\tau = \tau o + \gamma \eta \tag{1}
$$

$$
\gamma^{0,5} = \gamma \sigma^{0,5} + \eta^{0,5} \gamma^{0,5}
$$
 (2)

$$
\tau = K\gamma^{\eta} \tag{3}
$$

$$
\tau = \tau \mathbf{o} + \mathbf{K} \gamma^{\eta} \tag{4}
$$

The consistency index and flow index were determined through the Ostwald (3) flow model, where $τ$ is the shear stress, K is the consistency index, γ is the shear rate, and η is the flow index obtained from the equation, which describes the log of the shear stress vs. the log of the shear rate.

Discriminative and affective sensory analysis with consumers

The initial evaluation was performed through discriminative sensory analysis of samples N and L, with 88 consumers, men and women, aged between 17 and 40 years, with no previous training. The protocol presented in this study was approved by the ethics committee. People with sleep disorders, previous cases of allergy or health problems, pregnant women and smokers were excluded from this research. After presenting the research to them, they signed the terms of informed consent, and the discriminative sensory analysis was conducted according to the paired comparison experimental design.

Each participant evaluated a pair of samples, in triplicate (total of 6 samples). The sample pairs and the order of presentation were random, and thus, the volunteers could evaluate samples which were equal or different from each other. The possible combinations were as follows: N-N $(n = 61)$, L-L $(n = 63)$ or N-L $(n = 140)$. These n are related to the number of evaluations for each pair of samples. The samples were identified with 3-digit alphabetic codes. In each replicate, the codes were modified to avoid that the volunteer could identify the samples by the given codes.

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The volunteers washed their hands and forearms, up to the elbow, with neutral soap without perfume. Circles of 51 mm diameter were drawn on each forearm using an open mould. The samples (0.3 g) were applied by volunteers in the circles on the right forearms using the left hand and vice-versa and evaluated in pairs. The consumers were questioned as to whether they could note differences between the samples considering the following attributes: spreadability (ease of distributing the product over the skin), stickiness (intensity of finger adherence to the skin), oiliness (greasy sensation on the skin) and sulphur odour (typical unpleasant smell of sulphur). 20 min after product application, the residual attributes were observed, except for the spreadability attribute, which was evaluated immediately after application. The experiment was performed in a room at 20°C, and 60–70% relative humidity.

The affective sensory analysis was conducted together with the discriminative sensory analysis. Therefore, when the consumers answered that they could perceive a difference between the samples with regard to one of the attributes, they were asked which sample they preferred in relation to that attribute.

Statistical analysis

A comparison between the preferences of the consumers was performed by a two-proportion bilateral test. The statistical software used was XLSTAT 2009 for Windows. The rheological data were evaluated applying the Kruskal–Wallis test, using the program Statgraphics 5.1, and 95% was considered the significance level in all analyses.

Results and discussion

Physicochemical and rheological characterization of the formulations

The nanocapsule suspensions presented an average particle diameter of 313 ± 2 nm (polydispersion index of 0.24 ± 0.03), zeta potential of -6.05 ± 0.06 mV and encapsulation efficiency of 79%. The semisolid formulation containing the nanocapsules had a lipoic acid content of $102.9\% \pm 3.47$ and pH value of 5.62 ± 0.04 , while the formulation containing free lipoic acid had a lipoic acid content of $123.31 \pm 5.5\%$ and pH of 5.47 ± 0.02 .

The formulations presented pseudoplastic flow, according to the rheogram (Fig. 1), and the coefficient of determination was higher

Table II Consistency index (K) and Flow index (η) of formulations

Formulation	к	η
	476.92 ± 18.44 570.84 ± 23.56	0.295 ± 0.003 0.258 ± 0.004

The samples N and L presented a statistically significant difference $(P = 0.0495)$, Kruskal–Wallis test, significance level of 95%.

than 0.99 for both formulations when the data obtained were adjusted to the Ostwald flow model (N and L). Pseudoplastic flow was maintained, independent of the incorporation of the nanocapsules.

By means of Brookfield viscometer, with the suitable spindle, it is possible to obtain the viscosity values of non-Newtonian fluids in a trustable way. This method has been employed to determinate the rheological profile of different types of formulations, including emulsions [33–37]. It is important to notice that the viscosity obtained was not a constant value, but it changed according to the shear rate, as seen in Fig. 1. For Newtonian fluids, the viscosity is not dependent on the shear rate applied to the sample, whereas for non-Newtonian fluids it is. So, different viscosities are observed for different shear rate values, and a graph can be obtained with such values. However, when absolute values are needed, which represent the sample independently from the shear rate, the consistency index and flow index can be obtained, by fitting the data to the Ostwald flow model. Then, by obtaining such values (consistency index and flow index), it is possible to compare the formulations.

The flow index was less than 1 for all formulations, confirming the pseudoplasticity of the formulations (Table II)[38]. The consistency index decreased while the flow index increased when the nanocapsules were added ($P = 0.0495$) (Table II). Thus, the formulation containing the free active compound (L) presented a significantly higher degree of pseudoplasticity.

Discriminative sensory analysis of the formulations

In discriminative sensorial analysis, the focus is the difference between specific attributes of different samples. It is possible that two samples correspond to different formulations, although the differences are not noticed by the consumers [39, 40]. Through discriminative tests, it is possible to verify whether there is a difference between the samples tested, enabling the evaluation of specific attributes of different samples and obtaining an overview of the products. However, there is no quantification of the extent of the attributes [41]. Therefore, a high level of training of the evaluators is not required, and non-trained panellists can also participate [39, 41, 42].

In the discriminative sensory analysis of the products, using the 'paired comparison' experimental design, it was observed that the consumers presented a tendency to note differences between the samples, even when equal samples were presented (data not shown). The percentage of right answers was close to 50%, considering a right answer when the volunteers realized that the samples were the same. This result is explained by the fact that the volunteers felt forced to make a choice [43]. It is described in the literature that sometimes consumers can imagine a difference when none is observed. This result may also reflect the use of consumers Figure 1 Rheological properties of formulations N and L in such evaluations who were not previously trained [39, 44].

When different samples were evaluated by the 88 consumers in the paired comparison experimental design, the samples N and L were statistically different in terms of spreadability, stickiness and oiliness (Table III). In the paired comparison tests, where the consumer perception is evaluated regarding specific attributes, it is considered that making a choice, for more than 55% of the volunteers, excludes the possibility of equality between the samples [21].

The spreadability, stickiness and oiliness are the attributes, which the panellists noted a difference with statistical significance. The difference found in the spreadability may be explained by the results found in the rheological profiles and consistency index between formulations. The direction of the preference is considered in the affective sensory analysis approach discussed later in this report.

The number of responses, where a difference was detected in the immediate and residual sulphurous odour, in the residual stickiness and in the residual oiliness was not statistically sufficient to affirm that the samples were different in such attributes (Table III). Thus, it is considered that, in general, the samples N and L did not present noticeable differences to consumers regarding sulphur odour and any of the residual properties. So, the sensory changes did not compromise the residual attributes, which may have a higher impact for the consumer continuous adherence to the treatment.

Previous works have shown possible benefits from nanotechnology, such as higher stability and improvement in the antioxidant activity of lipoic acid by its nanoencapsulation [30, 45]. Also, the literature reports advantages in the nanoencapsulation of several substances, for example controlling delivery, higher cutaneous tolerance and improvement in efficacy [12, 13, 16, 30]. Nevertheless, the present results show for the first time, that nanotechnology can change some sensory characteristics of the formulations like spreadability and stickiness.

However, it is important to consider that non-trained volunteers may not be completely able to apply the technical language used to describe the attributes [46]. On the other hand, even different samples can have similar performance regarding some attributes [43].

Table III Discriminative and affective sensorial analysis: comparison between samples N and L $(n = 140)$.

*Represents statistically significant difference with at least 95% confidence.

Affective sensory analysis of the formulations: linking sensory characteristics and rheological profiles

In the affective sensory analysis, also called affirmative or hedonic, the level of acceptance of a product is evaluated with a focus on the preferences of the evaluators [40, 47]. This experimental design is more common in tests with consumers and in marketing studies [5]. The restriction of this test is that, in general, it does not evaluate the magnitude of the difference between the samples [39]. This technique is considered less reliable and reproducible, because two different samples can be equally accepted for different reasons. A consumer might know which product is the preferred one, without knowing how to express this preference [48]. Acceptability and preference tests can be applied in the early stages of the development of a cosmetic or to aid in the definition of standards and sensory specifications related to the level of consumer acceptance. It is necessary to have a high number of evaluators, with around 75 to 150 people commonly being recruited, and inclusion of consumers that use the type of the product tested, to compensate for the high variability in the individual preferences and to guarantee the statistical power and the sensitivity of the method [39].

In this study, on comparing products N and L, some consumers demonstrated higher discriminative capacity, and were able to detect differences between the attributes and the samples. Thus, exploratory analysis was performed to evaluate the preference of the consumers who noted differences for each attribute evaluated.

As the sensory characteristics of cosmetic products are related to their rheological properties, it is useful for the sensory analysis to be performed together with the rheological analysis. Although this relation between the rheological and sensory properties has been previously described, it has been little explored [23, 49]. The flow properties determine the consistency and spreadability of cosmetic products[50] and thus are related to the sensory characteristics [51]. As presented in Table III, the consumers preferred the formulation containing free lipoic acid (based on spreadability and residual oiliness), which exhibited higher consistency index and a lower flow index. Consumers perceive formulations that have higher consistency index to be better hydrating, richer and more concentrated systems, which may explain their preference for a more consistent product in terms of these attributes [52, 53]. The capric/caprylic triglycerides compose the oil core of nanocapsules, which is surrounded by a polymeric wall [54], and thus, protected from direct contact with skin in the formulation N. On the other hand, in the formulation L, the capric/caprylic triglycerides are not protected by a polymeric wall, leading to greater contact between the skin and the triglycerides. This may be the reason for the perception of higher residual oiliness regarding formulation L in the affective analysis, considering the consumers who noted some difference in this attribute.

The sensory properties perceived during the product application are more strongly related to the rheological properties, like spreadability, although the emollient ethers contribute to the residual sensory properties, like residual oiliness [52]. The sample containing nanoencapsulated lipoic acid (N) was preferred in terms of the immediate stickiness attribute and residual sulphur odour (Table III).

Considering the immediate sulphur odour, the consumers were not able to detect significant differences, probably because at the moment of application the odour was more intense, and it was

 \odot 2012 Society of Cosmetic Scientists and the Société Française de Cosmétologie International Journal of Cosmetic Science, 35, 105-111 109 difficult for non-trained volunteers to distinguish between the samples. However, the sample containing nanoencapsulated lipoic acid (N) was significantly preferred regarding the residual sulphur odour among the consumers who noted a difference. This difference is probably due to the nanoencapsulation of lipoic acid, which reduced the evolution of volatile sulphur odour and protected the active substance from degradation (related to the accentuation of the sulphur odour). It is important to mention that the samples were used right after preparation, and no degraded samples were used. It is possible that the differences would be emphasized after sample ageing [29–31].

This kind of sensory analysis is essential in the development of products because it takes into account the consumer preferences [46]. However, the data can be overestimated or underestimated, because analysis by consumers is highly subject to mistakes and may not be sufficient to make conclusive inferences regarding the differences and similarities between samples. Different samples can be equally preferred for different reasons. The consumers can express their tastes and preferences, without, however, giving information regarding the reason for preferring a particular sample [46, 48].

Conclusions

The sensory perceptions arising from the cutaneous application of cosmetic products play an important role in product development and quality control. These characteristics are as relevant

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to consumers as the product's efficacy and safety. Furthermore, the sensory methodologies are accepted measures to substantiate claims. The motivation behind buying and using a product is certainly influenced by its sensory properties. Apart from the intended application, it is essential that the objectives of the sensory studies are clearly defined in advance and that the experiment is carefully prepared for reliable results to be obtained from the panel.

Considering the study described here, it was verified that the sensorial perception of consumers is related to the rheological properties and that the pseudoplastic flow is maintained, regardless of the presence of nanocapsules. The discriminative analysis showed that nanotechnology can change some sensory characteristics of the formulations, such as spreadability and stickiness. Regarding the affective analysis, which considered consumers who noted differences for each attribute, the sample containing the nanoencapsulated active compound (N) was preferred when evaluating immediate stickiness and residual sulphur odour; whereas the sample containing free lipoic acid (L) was preferred for the attributes spreadability, immediate and residual oiliness.

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