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Spectrocolorimetry in the CIE *L*a*b** color space as useful tool for monitoring the ripening process and the quality of PDO red-smear soft cheeses

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Abstract

Smeared cheeses like Epoisses, Munster, Maroilles, Livarot, Limburger or Tilsit are characterized by the occurrence of red to orange-brown surfaces. The rind's color of these cheeses originates in the synthesis of carotenoids and other pigments by bacteria such as *Brevibacterium linens* group and coryneform bacteria, in interaction with deacidifying yeasts and cheese technology. Objective measurement of the smear color can be provided using a spectrocolorimeter (*L*a*b** colorimetric system). As a means to bring information to cheese manufacturers making so-called red-smear soft cheeses, the use of spectrocolorimetry was investigated for the description of: (i) the rind's color among various PDO cheeses, (ii) the relative heterogeneity observed for "on shelf cheeses" color in a specific PDO area (example taken from Munster produced in France), (iii) the assessment of color development at the surface of cheese versus time (quality control), (iv) a screening technique for pigmented strains isolated from red-smear cheese rinds (biodiversity criteria).

Keywords: Spectrocolorimetry; Red-smear cheese; Color; Ripening; Microbial flora

1. Introduction

The appearance of foodstuffs is the only permitted way to appreciate on-sell food products. In that respect, color is a clue for many qualities of food such as flavour, sanity, naturality or maturity, and drives consumers' choices. An attractive aspect is therefore a key for food marketing, and this has led the food industry to devote much effort in offering pleasant and suggestively colored products.

For so-called red-smeared cheeses like Maroilles, Munster, Livarot, Epoisses, Limburger, Herve, Gubbeen, Taleggio or Tilsit, a good aspect is mostly characterized by the occurrence of an orange-brown, sticky surface (Table 1). Since it is due to the development of cheese-ripening flora and to the interactions within this microflora [especially bacteria from the *Brevibacterium linens* group (Gavrish et al., 2004; Guyomarc'h, Binet, & Dufossé, 2000a), *Arthrobacter* sp., *Microbacterium* sp. (Brennan et al., 2001a) and coryneform bacteria], these typical dark yellow, orange, pink or red-brick colors related to carotenoids such as isorenieratene (Savy & Dufossé, 2002; Valla et al., 2003) and other pigments ensure that the cheese has developed aromas and melted texture. The ripening of cheese is a very complex, and often slow, biochemical process that involves three primary reactions: glycolysis, lipolysis and proteolysis. It is a relatively expensive process and there are economic and technological incentives to accelerate the rate of

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Table 1
Details on smear cheeses produced in France

Name of cheese	PDO agreement	Production in 2003 (tons)	Comments
Epoisses	1991	796	Lactic curd. Rind is shiny and smooth or slightly rippled, orange-ivory to brick-red in color depending on its degree of maturity. The color is due solely to the pigmentation of the surface bacteria, the use of color additives being prohibited. Ripening time: at least four weeks.
Langres	1991	335	Curd is molded and successive washing operations take place in a cellar during maturing, which may last from 15 days to 3 weeks, with the possibility of being extended to 3 months in a humid cellar. The rind is colored with a plant-based dye extracted from the annatto tree, a South American plant. The dye gives the cheese a distinct yellow-orange to brick-red appearance.
Livarot	1975	1269	Livarot is one of the oldest cheeses in Normandie. The cheese is circled by five bands of rush leaves that prevent the cheese from collapsing during maturing. In the course of its maturing, Livarot is colored reddish-orange with the natural taint of annatto. Four weeks of ripening.
Maroilles	1976	2346	The full-size Maroilles is square and weighs 800 grams. It has a washed rind, which can be further described as soft, smooth and shiny. It is brushed and turned regularly in order to obtain the natural light-yellow to red brick color of the rind. 2 to 3 months are required for perfect maturing.
Mont d'Or	1981	3764	The surface of the cheese is moist, with a rind that is golden and slightly reddish. Inside is soft yellow and creamy. The cheese is always presented in a wooden box and bound with spruce hoops. These should never be removed, even when serving, as they enable the cheese to be contained.
Munster	1969	7239	Maturation at a temperature of 12–14 °C. This takes 4 to 6 weeks for small thin cheeses and 2 to 3 months for thicker larger cheeses. Every two days the cheeses are washed and brushed with brine and in some cases with annatto. The paste is soft and creamy and has a shiny brick red, pink, orange or dark yellow rind.
Pont-L'Evêque	1972	3195	Firm square body, yellow color and edible rind. The rind has ridges because it is cured on straw mats. Pont-l'Eveque has a slightly mouldy, pink to brown rind and a soft, supple paste. The cheese must be regularly washed, brushed and turned to encourage the special bacteria to grow at the surface.
Reblochon	1958	16,987	Cheese is stocked in a cellar where it will age for some two to four weeks at a temperature not to exceed 16 °C. Each cheese must be turned over every two days during the aging process. Once ripe, the cheese has cream-colored soft to firm paste surrounded by a light beige to orange rind.

maturing and to reduce costs (Ferraza, Fresno, Ribeiro, Tornadijo, & Mansur Furtado, 2004). Moreover, as knowledge of the representatives of the cheese-surface bacterial flora is quite limited (Irlinger, Bimet, Delettre, Lefèvre, & Grimont, 2005), industrial manufacturers may sometimes encounter quality problems and the sole ripening pigmented microflora is not sufficient to give a nice color. In this case, colorants such as annatto, paprika or β-carotene are spread at the surface of the cheese during processing, as a means to produce standard and attractive products. This situation was also described in the industrial production of Turkish Kashar cheese, where color intensity of the product does not meet the consumers' acceptance (Öksüz, Kurultay, & Simsek, 2001). The objective of the present work was to investigate spectrocolorimetry, an analytical technique which is not well introduced in the dairy industry, for the description of various items useful for improving the ripening process of high quality red-smear soft cheeses such as those under the "Protected Designation of Origin (PDO)" legislation, i.e., the adjunction of colorants is forbidden or avoided in some PDO cheeses and the color development should only be related to the occurrence of a well-balanced microflora.

Another aspect is related to food authenticity and geographic origin. The originality of a cheese depends on several factors such as milk and cheesemaking procedures (including microbiology and technology), which are both dependent on the geographic origin. The climate, geology, forage and breed itself, influence the cheesemaking. The determination of origin is a key component of PDO products (Pillonel et al., 2002). The present colorimetric studies will contribute to a better description of PDO red-smear ripened cheeses.

2. Materials and methods

Color measurements were performed on a CM-3500d spectrocolorimeter (Minolta, Carrières sur Seine, France),

driven with a SpectraMagic 1.01 software (Minolta). Reference illuminant was D65 (standard daylight) and geometry was d/8: incident light was diffuse and observation angle was 10°, according to the CIE 15.2 publication and ISO 7724/1 recommendations. Data were reported in the CIE $L^*a^*b^*$ colorimetric system. For cheeses, 10 to 14 measurements were made on the upper and lower sides. For microorganisms, following the cultivation step according to (Guyomarc'h, Binet, & Dufossé, 2000b), a single-pieced disk of agar was cut from the solid cultures in a place supporting most homogeneous cell development. The sample was then held culture-down and layered at the bottom of a 45 mm diameter CM-A128 glass Petri dish (Minolta). The pigmented bacteria covered face of the agar thus faced incident light during color measurement of the cultures. All bacterial samples were analysed in one single session, 5 replicates per sample.

3. Results and discussion

As a means to bring various information to cheese makers, spectrocolorimetry applications were taken in the following situations:

- analysis of the rind's color among various PDO cheeses,
- description of color heterogeneity observed among cheeses processed within a PDO area,
- assessment of color development on cheese rinds versus time,
- screening technique for pigmented strains isolated from the cheese surface.

3.1. Color distribution among various PDO cheeses

The parameters a^* and b^* presented considerable differences (Fig. 1). A first group of less colored cheeses, including Mont d'Or and Pont-L'Evêque, could be identified in the down-left part of the figure. These products were more yellow than red, covered in part by a downy white *Geotrichum candidum*. The most colored cheeses were spread in the yellow-orange, orange and pink areas. Most reddish and most yellowish cheeses were Munsters. For some cheese plants, color data were closely grouped (Munster, plant A), less grouped (Maroilles, plant B; Munster, plant C) or quite spread (Maroilles, plant A; Munster, plant B). Maroilles from both plants appeared dark orange.

Very few studies have been published on cheese and color. Pillonel et al. (2002) tentatively linked the color of Emmentaler cheese to the use of silage type (grass silage versus maize silage). A significant effect of ripening time on b^* values was reported in cheeses made from raw, pasteurized and high-pressure-treated goat's milk (Buffa, Trujillo, Pavia, & Guamis, 2001). Öksüz et al. (2001) described the inefficacy of *B. linens* addition in the coloration of Turkish Kashar cheese. Pink coloration on the surface of blocks of

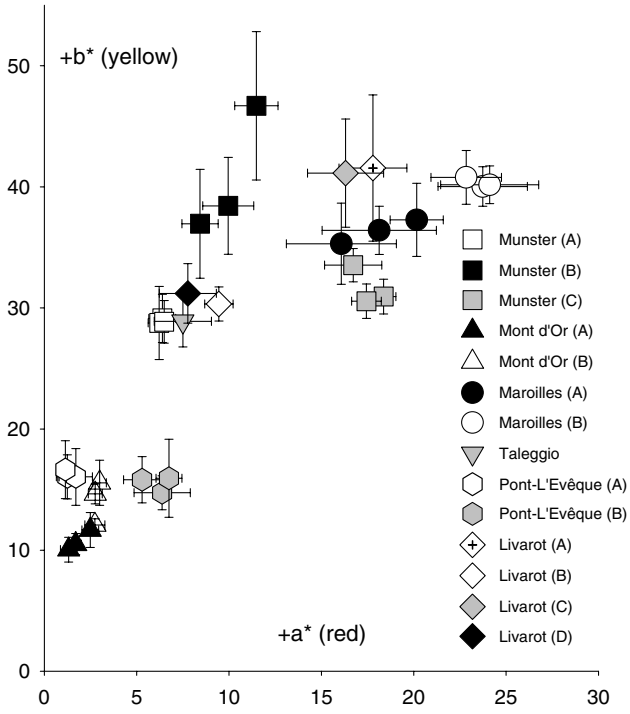

Fig. 1. Color distribution among various PDO cheeses (10 measurements per cheese, letters A to D: cheese manufacturing plants, one distinct letter series within one PDO).

Cheddar cheese was monitored by Martley and Michel (2001). In all cases, spectrocolorimetry was assumed to be a rapid and easy tool to assess these phenomenons.

3.2. Relative color heterogeneity among cheeses processed within a PDO area

Red-smear ripened soft cheeses are high added value products which have been manufactured for centuries all over Europe and further away (e.g., Epoisses, Livarot, Munster, Maroilles, etc., in France; Tilsit, Limburger, Muenster, etc., in Germany; Taleggio in Italy; Gubbeen in Ireland; Herve in Belgium, etc.). However, to our knowledge, there is no clear description in the literature about the color reproducibility of those distinctive PDO cheeses (Di Cagno et al., 2003). Munsters were collected from six French producers (3 cheeses per producer) and the $L^*a^*b^*$ coordinates (14 replicates per cheese) measured. As shown in Fig. 2A, the rind color of Munster is not unique, some are spread close to the yellow axis as others are more reddish. Consumers may face quite different products on store shelves, ranging from pale yellow, to dark orange or pink. Here an important question can be raised. Protected Designation of Origin within the European Union is a term used to qualify foodstuffs which are produced, processed and prepared in a given geographical area using recognised know-how. Is it sufficient or should these products be better described in order to ensure a target quality for on

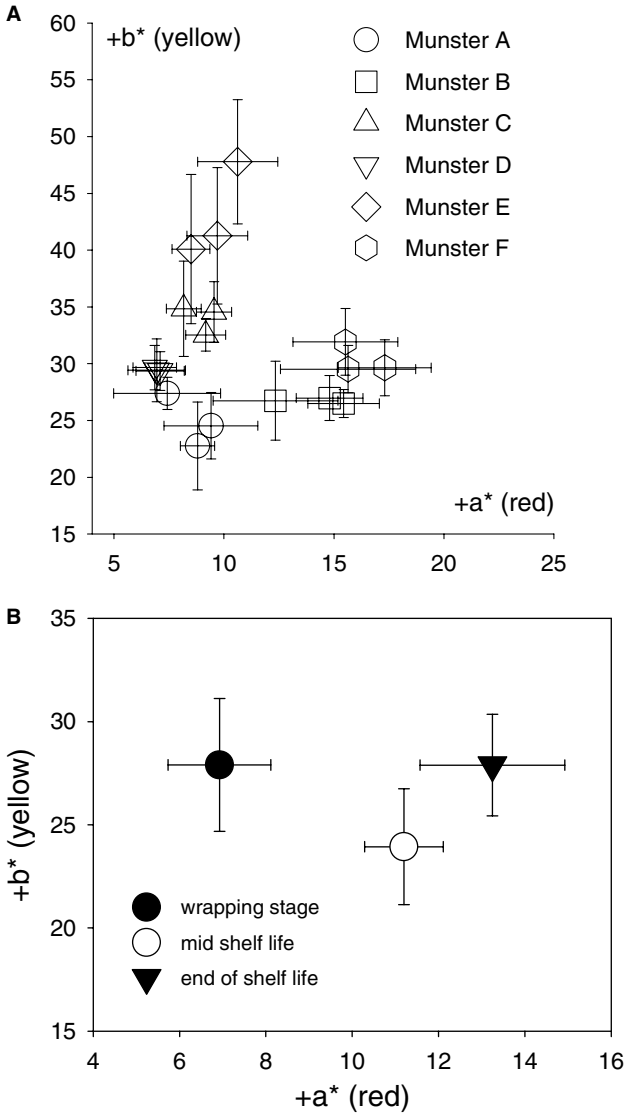

Fig. 2. (A) Position of Munster cheeses from six different producers in the CIE $L^*a^*b^*$ colorimetric system (3 cheeses per producer, 14 measurements per cheese). (B) Development of rind color versus ripening time (wrapping stage, mid shelf life, end of shelf life).

shelves products? Another point of view is to accept these variations as the expression of farmhouse-type productions. It may seem important to keep microbial biodiversity in PDO cheeses, each farm or small factory, helped by its distinctive microflora, could be able to produce high quality products with a total respect of specific know-how and cheese technology, in the frame of food safety criteria. Using the same starters and ripening flora within the global PDO area could reduce the overall quality of red-smear ripened soft cheeses.

3.3. Assessment of color development on cheese rinds versus time

The second illustration deals with the assessment of color development on cheese rinds versus time (Fig. 2B). This could be useful in many cases, e.g., controlling product quality in cheese plants, monitoring process modifications, R&D assays with new microorganisms used for ripening, or seasonal effects, etc.

Munster cheeses were monitored from the wrapping stage to the end of shelf life (Fig. 2B). Beyond the overall global orange color, the yellow component of cheese rind is obtained quite quickly, whereas the redness continues to increase during the shelf life time (major shift of hue along the red axis).

3.4. Screening technique for pigmented strains isolated from the cheese surface

Up to now very few pigmented bacteria are available as commercial cultures for red-smear cheese manufacturing. Traditionally, growth of the surface microflora is initiated with the help of mature cheeses, which release part of their microorganisms into the brine used to wash the young curds. Only about 15 different strains of *B. linens* group are present on the market with various orange hues (Guyomarc'h et al., 2000b). Intensive use of these strains may contribute to a decrease in microbial diversity observed within the PDO cheeses, contributing to the loss of typicity for products proposed to European consumers. Bacteria belonging to other species than *B. linens* group are also lacking in commercial offers made by microbial culture companies and this should be corrected as many authors emphasized their contribution in the ripening of smear cheese rind, e.g., *Microbacterium gubbeenense*, *Corynebacterium casei*, *Arthrobacter bergerei*, *Arthrobacter arilaitensis* (Brennan et al., 2002; Feurer, Irlinger, Spinnler, Glaser, & Vallaeys, 2004; Feurer, Vallaeys, Corrieu, & Irlinger, 2004).

In order to be able to supply new pigmented strains to cheese producers, a screening experiment was conducted for two years and 364 strains were isolated from Munster (219 coryneform bacteria, 32 *Micrococcus*, 30 *Staphylococcus* and 83 *B. linens*). Color coordinates were determined for each of them and the two dominant colors were orange and yellow (Fig. 3. for a sample of 29 strains projected within the $L^*a^*b^*$ system). All the orange strains were easily identified as strain members of the *B. linens* group (positive reaction with KOH, HPLC analysis of the pigments, 16S DNA sequencing). The technological important group of yellow pigmented coryneform flora of the smear cheese is quite homogeneous in colony morphology and physiological characteristics. As biochemical identification methods are of limited value, Hoppe-Seyler et al. (2003) applied Amplified Ribosomal DNA Restriction Analysis (ARDRA) to identify yellow strains such as *Arthrobacter nicotianae* or *M. gubbeenense* which could be present in our isolates. Besides orange and yellow, a lot of strains were light-colored (cream, beige) as *Corynebacterium mooreparkense*

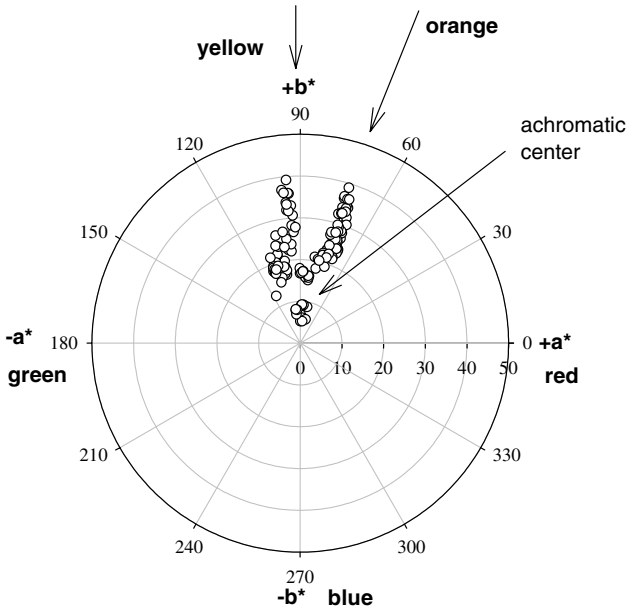

Fig. 3. Position of 29 pigmented bacteria isolated from Munster cheeses in the CIE *L*a*b** colorimetric system.

sp. nov. described by Brennan et al. (2001b), five were pink but a total of 29 hues were described.

The non-orange isolated strains are currently under study: 16S DNA sequencing, HPLC analysis of the pigments, pilot scale cheese-making with pure strains or bacteria-bacteria or bacteria-yeast combinations, etc.

4. Conclusion

Following the publication of our first papers dealing with the use of spectrocolorimetry in color development during cheese ripening (Dufossé, Mabon, & Binet, 2001; Guyomarc'h et al., 2000b), some colleagues successfully applied this technique for the demonstration of interactions within the bacteria/yeast microflora (Leclercq-Perlat, Corrieu, & Spinnler, 2004; Leclercq-Perlat, Spinnler, & Corrieu, 2001; Masoud & Jakobsen, 2003). In the initial stages of ripening, yeasts such as *Debaryomyces hansenii* constitute a major part of the surface microflora of red-smear cheeses and contribute to ripening by assimilation of lactic acid causing an increase in pH, which enhances the growth of pigmented coryneform bacteria. *D. hansenii* and other yeasts have a significant effect on the intensity of the orange-reddish color and differences among strains were observed, based on *L*a*b** measurements of bacteria/yeast combinations.

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