Colour of milk and milk products in CIE L*a*b* space

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Summary

Colour measuring instruments have become more reliable, smaller and faster than their predecessors at significantly lower costs for the users. This article has been written to provide practical guidelines for technologists, technicians and scientists to better understand colour measurement of dairy products. The results of milk colour measurement are used to determine the health of cows, as well as the quality of the raw material. The measurement of the colour of final dairy products makes it possible to determine their quality and to monitor changes occurring during storage.

The article presents the theory of colour evaluation in the CIE L* a* b* space. It specifies what conditions need to be fulfilled while determining the colour and what information should be given when publishing data on colour. The article explains what affects the colour of milk. The article gives examples of studies on the colours of dairy products and practical applications of such studies.

Keywords: CIE Lab, colour, dairy products, milk

Since the colour of objects is a feature originating in human mind, the perception and interpretation of colour remains subjective. An illuminated object reflects light energy, either partially or completely. A reflected electromagnetic wave reaching an observer is captured by the eye, and, after passing the signal to the brain, an impression of colour is generated. An analogous phenomenon is observed during sunbathing. People cannot see solar radiation, but can feel it warming them up – thus an impression of warmth is created in their minds (also subjectively). Since our bodies differ, we experience impressions in different ways. The reception of colours depends on the eye structure, educational background, natural environment and mental aptitude (27, 42). A mood or illness can also affect colours created in the brain. Due to disturbances in the eye or brain structure, a certain percentage of society is unable to generate the whole range of colours. Thus, as an instrument of colour evaluation, man is not free from drawbacks.

An objective colour description is indispensable, particularly wherever slight differences in colour are significant, as in textile, paint and foodstuff industries or in dentistry (22). Nowadays, spectrophotometers are most frequently used for a quick evaluation of product colours. They convert energy reflected from an object into numbers defining the colour. The space for the

expression of product colour is nowadays usually the CIE LAB colour space. In this space, the colour is expressed by means of three coordinates (constituents): L*, a*, b*. For better visualisation of the results, constituent L* can be presented as an independent vertical line with the following values: 0 – black, medium values – greys, and 100 – white. Values a* and b* can be presented as a point in the ab coordinate system, that is, on a colourful plane where colours fluently pass from red (+a*) through yellow (+b*) to green (–a*) and blue (–b*), just like the colours of the white light spectrum after passing through a prism. By connecting a point with coordinates a*b* with the centre of the coordinate system, a segment line is obtained, whose length is defined as chrome (c). The higher the value of c, the more intense (stronger and clearer) the colour of the object, and vice versa: the lower the c (closer to the centre of the system), the paler grey the colour is. The angle created by the axis +a* and the line segment c is called the hue (i.e. colour), and denoted by the letter h. The parameters obtained while measuring colour depend on spectrophotometer settings: the source of light (31), the angle of light incidence and observation (measurement geometry), the observation angle of the observer (2° or 10°), the diameter of the measurement area, and, for thin layers of samples, the parameters depend on the surface colour (3, 45). The

most preferred source of light is the equivalent of day-light, marked with the symbol D65. The 10° observer was developed as an improvement on the original 2° (30). When describing research, it is recommended to give information on the model according to which the colour measuring device was calibrated. Most spectrophotometers used for measuring light in a colour management system have an opening with a diameter of 4-8 mm. The bigger the opening, the lower the influence of surface differences on the measurement. When describing research on liquid and loose products, the type of the tray should be given, and it should be specified whether the measurement was made through the tray glass.

Preparing the sample is a fundamental problem of colour measurement. During the analysis of stable samples, it should be remembered that

- the sample should be flat (for dispersed light to be homogeneous),
- the structure of subsurface layers and dustiness affect light dispersion,
- the structure of pigments depends on the temperature of the product examined,
- the measurement opening needs to be adjusted to the size of the sample (the sizes of the opening and the sample need to be given),
- light sources in measuring devices are very intense, so the type of surface on which the sample is placed can affect measurement results,
- dry samples are darker than those with an increased water content,
- the number of repetitions needs to be calculated for the material tested,
- the description of samples should include their chemical content.

Cheng at al. (9) demonstrated that also the temperature of the sample and the measurement system (CIE, Hunter) should be specified.

In their research, Chudy et al. (10) calculated the necessary number of repetitions, depending on the adopted measurement error. An x-Rite spectrophotometer (SP60) was used in this study with the following settings: measuring geometry – d/8, measurement opening – 8 mm, normalized light source – D65, normalized observer – 10°, setting option SPEX. For a 2% error, the number of repetitions, depending on the system, was as follows: CIE Lch – 10, CIE Lab – 13 for ripening rennet cheese; CIE Lch – 2, CIE Lab – 33 for butter; and CIE Lch system – 9, CEL Lab – 22 for processed cheese. The most repetitive parameter describing the colour of processed cheese was h (hue). Even for a 1% error level, only 2 repetitions were required for ripened cheese and processed cheese, and one repetition for butter. The authors determined that, depending on the size of indication error, the lab/researcher should decide on a different number of repeats which need to be performed when defining product colour. If the

indication error is to be small, e.g. 1%, more repetitions are required for the results to be reliable.

Visual comparison of colours is based on differences in hue (h), intensity/chroma (c) and lightness (L^*). The human eye can see differences in hue easier than those in chroma. Therefore, the most stringent criteria are those pertaining to the difference in hue. With an increase in c, the ability of the human eye to capture differences in the constituents of colour (L^* , a^* , b^*) increases. Most often, the correlation of lightness, intensity and hue is assumed as the ratio of $L^* : c : h = 4 : 2 : 1$ (34). The L^* , a^* , b^* values can be used to calculate the value of colour difference (ΔE) for comparison between two samples, $\Delta E = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{0.5}$. Numerical values of the accepted colour deviations should, nevertheless, be varied, depending on colour, intensity and product type (26).

Factors affecting milk colour

The appearance of milk is white. It results from its physical and chemical structure (38). The natural colour of milk is due to the reflection of light by dispersed fat globules, calcium caseinate and calcium phosphate. Milk also contains two classes of pigments: water-soluble and fat-soluble ones. The water-soluble pigment, which imparts a yellow colour with green fluorescence to the whey of milk, was called lactoflavin. It is better known as riboflavin or vitamin B₂, but is also referred to as vitamin G or lactochrome (19). Milk is relatively rich in this vitamin. The riboflavin content in cow's milk ranged from 1.16 to 1.31 $\mu\text{g ml}^{-1}$. Riboflavin is heat stable and light sensitive. For cow's milk stored in opened containers in a refrigerator at 8°C (in the dark), the loss of riboflavin ranged from 16.0% to 23.4% (37). A fat-soluble pigment found in fat gives fat-rich milk products a more or less yellow tinge. The depth of colour depends on the amount of pigment present. The group of pigments called carotenoids includes β -carotene, retinol and xanthophylls (i.e. lutein and zeaxanthin). The colour of carotene varies from yellow to orange and deep red-orange as the concentration increases. The amount of carotene in butter oil depends on the amount of carotene in the food of cows (2). Carotenoids are synthesized in plants, but not in animals. Green grasses, hay carrots and corn are rich in carotene. In their study, Mogensen et al. (35) found concentrations of 0.17 and 0.41 mg of β -carotene and retinol, respectively, per one litre of milk in five organic dairy herds in Denmark. Many factors have been suggested to explain the variability in carotenoids in milk, including non-dietary factors, such as breed, stage of lactation, health status of the udder, milk and fat yields as well as genetic traits (19). The yellow coloration is higher for Jersey cows than it is for Holstein or Montbéliarde cows. Also Guernseys are known for the deep yellow colour of the fat in their milk due to β -carotene retained from feed

(5, 15). Goat's and ewe's milk, in contrast, contains no β -carotene, only retinol and xanthophylls (41, 47). Goats and ewes convert β -carotene (from plants) into vitamin A, which lacks colour.

Milk can also be reddish or pinkish because of the presence of blood. There are several causes of blood in milk. Important causes of this disorder are haemorrhage, systemic microbial infections (several infections, including those caused by some bacteria, some viruses and red yeast), feeds containing natural toxins or dyes, deficiency of blood platelets and other causes (vitamin C deficiency, rough milking and acute or chronic mastitis) (36). Inflammation is characterised by changes in the composition and appearance of milk. Abnormalities in milk may include the presence of flakes and clots or watery appearance.

Cow's colostrum – the first secretion of the mammary gland after parturition (characterised by its high content of immunoglobulins, predominantly IgG) – can have a wide colour spectrum, from dark brown/red through yellow to pale white (20). Colostrum generally contains higher concentrations of β -carotene and retinol (23). In the course of a couple of days, colostrum turns into milk in its appearance and content.

Data (29, 46) show that the colour of milk can be changed by controlled feeding as well as by genetic manipulation of cows.

Colour of dairy products and practical application of colour evaluation in dairy industry

The yellow coloration of dairy products is generally more important in high-fat dairy products, such as butter and full-fat cheeses. Because carotenoids are fat-soluble, the yellow coloration is a function of both fat colour and concentration, and fat colour in its turn is a function of carotenoid concentration in fat (49). The colours of selected products are presented in Table 1. As can be seen, the natural colour of dairy products is situated in the 1st and 2nd quarters of the coordinate system with the a^* and b^* axes, which is indicative of the colour yellow. The yellow colour of products can be monochromatic (580 nm) or generated by a greenish (540-560 nm) and red (600-700 nm) radiation mixture.

Scientific publications frequently lack data on the light source of the device used or the measurement

geometry. One practical application of colour measurement was found by Everard et al. (17). They used colour parameters to monitor curd syneresis during cheese making. El-Nimr et al. (16) used colour measurement to monitor changes in colour and to determine correlations between sensory evaluation and colour parameters during the ripening of cheese.

Since the colour of the product affects its reception by consumers, research on how colour influences human senses is both important and interesting. Research results (8) indicated that, even with the same content of each fruit flavour and sugar, the greater the concentration of colorant, the greater was the intensity of taste perceived by the assessors in yoghurts with strawberry, orange and fruit of the forest flavours. With regard to the perception of sweetness, only in yoghurts with fruit of the forest flavour was a greater concentration of colorant associated with a greater sensation of sweetness. Rohm et al. (43) proved that sensory spreadability is heavily affected by butter colour. The more yellowish butter was selected and described by consumers as “easier to spread” (43). It can be concluded from research (16) that cheeses scored better in sensory evaluation with increasing a^* and b^* and with decreasing L^* . The colour of butter may vary from light, creamy white to orange yellow. Differences in butter colour result from variation in the colour of butter fat, variation in the size of fat globules, the presence or absence of salt, the condition of working butter (21), the type of packaging and storage temperature (1). The colour of dairy products also depends on the type and amount of colouring added. The European Union permits carotenes (E160a), annatto, bixin and norbixin (E160b) as additives to ripened cheeses, and 13 types of colorants, including lutein, as additives to processed cheese (12). Jones et al. (25) and Kubo et al. (28) experimented with adding lutein as colouring. The objectives of their study were to determine the transference of lutein additive (dye) from milk to whey and cheese, to evaluate the effect of lutein addition, light exposure and storage time on cheese colour and to verify the sensory acceptance of Prato cheese with addition of lutein. Technological processes, as well as conditions and time of storage, can result in colour changes in dairy products. Homogenisation causes an

Tab. 1. Examples of colours of dairy products expressed in CIE $L^* a^* b^*$ system

Product	L^*	a^*	b^*	Conditions	Source
Yoghurt (2% fat; pH = 4.40)	92.07	-2.64	9.14	10-12°C	(7)
Butter (83% fat, protein and carbohydrates 0.7% of each)	90.53	1.29	18.03	D65/10°, 8°C	(10)
Butter	91.6	5.5	24.7	D65/10°, 15°C	(43)
Maturing rennet cheese (27% fat, 26% protein, 1.2% carbohydrates)	77.17	8.25	32.16	D65/10°, 8°C	(11)
Mild Cheddar cheese (30.5-32.5% fat)	69.6	9.6	28.7	D65	(49)
Demineralised whey (75.6% lactose, 15.5% protein, 3.7% mineral salts, 1.1% fat)	95.46	-0.68	13.93	**D65/10°, 20°C	authors

Explanations: ** – measuring equipment: spectrophotometer X-Rite SP-60, glass cuvette; n = 10

increase in whiteness (cream, milk), whereas thermal processes may cause either an increase or a decrease in parameter L^* (6). An increase in lightness occurs due to denaturation of β -lactoglobulin and its conjugation to j-casein. Devi et al. (13) proved that coloured products from high-pressure thermal processing (HPTP) of skim milk come primarily from lactose degradation. The rates of colour changes and proteolysis increased with increasing temperature and duration of HPTP. The colour of milk changed drastically at 400 MPa, where most of milk proteins formed coagulates and left the solution nearly translucent. The colour of skim milk changed from white to caramel brown during HPTP at 400 MPa and 100, 110 and 120°C.

Popov-Raljić et al. (40) examined colour changes in UHT milk with a fat content of 3.2% stored at a temperature of $20 \pm 5^\circ\text{C}$ for 90 days. They noted changes in L^* from 89.88 to 77.15, in a^* from -3.26 to 2.12 and in b^* from 9.27 to 7.06 .

As shown by Chudy et al. (11), during storage of whole milk powder (26.4% of fat) at 20°C , both vacuum-packed powders and those packed in the environment of air became darker (L^* value decreased) by month 12 of storage and then lighter (L^* value increased) by month 24 of storage. The darkening of products in storage could be caused by non-enzymatic browning reactions (Maillard reactions, lipid peroxidation, degradation of ascorbic acid or sugar-sugar caramelisation). The reaction of the amino group of lysine and the carbonyl group of lactose is the main one in whole milk powder. Pentose or xylose undergo dehydration and, losing a water molecule, become furfural or hydroxymethylfurfural (HMF) (32). In fresh milk powder, lactose appears in an amorphous (i.e. non-crystalline) form. With an increase in water level (around 5-7%), there is a threat of lactose crystallisation. Forming crystals cause relocation of other components, thereby undergoing local densification and stronger interaction with one another. An increase in salt concentration at a given point of milk granule may lead to denaturing alternations in casein.

Colour changes occurring during the production and ripening of cheese were described by Johnson (24). Each phase of cheese – solid and liquid – contains material that reflects specific wavelengths of light and therefore different colours of products. Light reflected from some components of cheese may overwhelm light reflected from other components. This can change with time, and that is why the colour of cheese can change with cheese age. Proteolysis that occurs during ripening can transform casein into a more soluble state and can cause a decrease in whiteness. Beta-caroten is very stable, whereas xanthophylls are partially damaged and/or lost into whey during cheese making (49). The colour of cheese also depends on its acidity. Mozzarella was whiter at the pH of 5.2 than at 5.0, when translucent areas were observed (24). Changes in the colour

of Garrotxa cheese during regular ripening and at high hydrostatic pressure (HHP) were studied by Saldo et al. (44). The decrease in cheese lightness (L -values) during ripening was associated with the concentration of cheese components. The moisture content in cheese during ripening decreased by 32% in regular cheese and by 23.95% in cheese subjected to HHP. HHP treatment at the beginning of cheese ripening proved successful in accelerating the process. This treatment leads to increased proteolysis, a higher moisture content and a higher pH.

The metabolism of bacteria in cheese may lead to the formation of dicarbonyl compounds that react with amino acids. The reaction produces pink or brown pigments (Maillard browning). Bacteria, moulds or yeast may also produce other pigments. 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 the 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 an orange-reddish colour (14). *Arthrobacter nicotianae* give a reddish-brown colour to semi-hard smear cheese. Yellow and orange strains of coryneform bacteria form a major part of the surface flora of smear cheese. Yellow, orange or red pigment is also produced by *Brevibacterium linens* (4). Galaup et al. (18) isolated 364 strains from Munster cheese (219 coryneform bacteria, 32 *Micrococcus*, 30 *Staphylococcus* and 83 *B. linens*). Besides orange and yellow, a lot of strains were light coloured (beige or cream), and five were pink. Paul-Sadhu (39) proved a significant correlation between the number of bacteria in fluid pasteurised skim milk and the values of L^* , a^* and b^* obtained from colour measurement.

Colour coordinates, chroma and hue can also indicate changes in fat. As reported by Méndez-Cid et al. (33), the peroxide value exhibits high correlations with h (0.788), L^* (0.612) and b^* (-0.631) and smaller ones with c (-0.598) and a^* (-0.332).

The use of colour measuring devices can ensure an objective evaluation and control of colours. These devices can be used for precisely identifying and comparing the colours of products. Thanks to the measurement of colours, technological changes can be introduced to maintain the parameters of colours or to adjust the colours of products to customer expectations. It is necessary to provide detailed descriptions of the measurements conducted so that the experiments can be repeated and their results compared with others.

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