

# Color in Concentrated Milk Proteins with High Sucrose as Affected by Glucose Replacement

M.S. Pauletti, E.J. Matta, E. Castelao, and D S. Rozycki

## ABSTRACT

A model system of sucrose, whole dry milk powder, and distilled water was used to evaluate color development during heating. Sucrose replacement by glucose and moisture were the selected variables in a second-order central composite design. The main color response was the Kubelka-Munk index  $K/S$  calculated from reflectance colorimetric measurement using the Self-Backing Reflectance Transformation (SBRT) procedure. Browning reached a zone of maximum  $K/S$  when sucrose replacement increased. Additional replacement in the maximum zone did not increase browning, but produced an opposite effect decreasing browning, depending on moisture content. Chromophore group formation was higher between 40 and 75% of sucrose replacement by glucose and at 20-30% moisture content.

**Key Words:** heat-induced browning, concentrated milk, glucose, Kubelka Munk, optical properties

## INTRODUCTION

HEATING MILK AT HIGH TEMPERATURES PROMOTES THE DEVELOPMENT of brown color. The milk becomes darker as temperature and time increase and the effect is more noticeable for mixtures of concentrated milk and some sugars. Maillard and caramelization reactions are the main factor in color development and occur simultaneously, depending on type of sugars and reaction conditions (Buera et al., 1990; Pauletti et al., 1995). Browning occurs in the manufacture of certain dairy products such as dulce de leche (Moro and Hough, 1985). In this case, partial sucrose replacement by glucose provides technological and economic advantages. Color measurements of dulce de leche have been reported (Buera et al., 1990; Hough et al., 1991; Pauletti et al., 1992). Visually perceived color differences could be adequately expressed by the Kubelka-Munk index ( $K/S$ ) calculated from colorimetric reflectance measurements (Buera et al., 1990). To determine the Kubelka-Munk coefficient (the light absorption-scattering ratio in equation (1), Koukoulas and Jordan, 1997), or sample color in the CIE space, it is necessary to obtain  $R_\infty$ , a reflectivity value where the influence of the backing must be negligible (USA TAPPI Test Methods, 1996/97)

$$K/S = (1 - R_\infty)^2/2R_\infty \quad (1)$$

Usually, this can be achieved by increasing the thickness (volume) of the sample until no appreciable changes are noted in reflectance measurements. Then, light is not transmitted but internally reflected by the sample. Therefore,  $R_\infty$  is a "self-backing" reflectance. If a sample of "infinite thickness" is not available, direct measurement of  $R_\infty$  is not possible. In this case: the sample is small (relative to the batch), and the specific transmittance of the product can be high. There are

*Authors Pauletti, Castelao, and Rozycki are with Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, CC266, 3000 Santa Fe, Argentina. Author Matta is with Instituto de Desarrollo Tecnológico para la Industria Química (UNL-CONICET) and Instituto de Tecnología Celulosa (FIQ-UNL). Address inquiries to Dr. M. S. Pauletti.*

few solutions to this problem. Some researchers have used an exponential solution proposed by Wyszecki and Stiles (1987). CIE tristimulus instead of reflectance values have been used (Buera et al., 1990; Hough et al., 1991) and referred to by Calvo (1993), to calculate the Kubelka-Munk function (1) without using sample transmittance. The solution we proposed here is an indirect estimation of  $R_\infty$  using equations derived from Kubelka-Munk theory, particularly an equation given by Gurevič (Olf, 1982). First, Eq (2) and (3) are used for an adequate sample (reference sample) of known or measurable  $R_\infty$  and  $R_{t0}$  to estimate  $R_b$ :

$$t_r^2 = [1 - (R_{r0}/R_\infty)](1 - R_{r0}R_\infty) \quad (2)$$

$$R_{rx} = R_{r0} + (R_b t_r^2 / 1 - R_b R_{r0}) \quad (3)$$

where  $R_x$  = sample reflectance of unknown  $R_\infty$ , over the backing  $R_b$ ;  $R_b$  = nonblack backing reflectance;  $R_0$  = sample reflectance over a black backing of standard or no reflectance;  $t$  = sample transmittance; and the subscript  $r$  identifies the reference sample.

Second,  $R_{px}$  and  $R_{p0}$  of the unknown sample are measured both, respectively, with the same backing and optical geometry. Finally,  $R_{p\infty}$  is obtained from Eq (4) and (5). For colorimetric computation, the procedure must be repeated with three standard filters (450, 557 and 680 nm).

$$R_{px} = R_{p0} + (R_b t_p^2)/(1 - R_b R_{p0}) \quad (4)$$

$$t_p^2 = [1 - (R_{p0}/R_{p\infty})](1 - R_{p0}R_{p\infty}) \quad (5)$$

$R_b$  can not be measured directly, especially when the backing is non zero depth and a part of a cavity that contains a 3D sample (Olf, 1982). The reference can be any kind of material that fits the classic Kubelka-Munk equation (1) and conforms to the container (the cavity). Once the reference is evaluated and  $R_b$  calculated, only  $R_{px}$  (reflectance over backing  $R_b$ ) and  $R_{p0}$  must be measured for each filter provided the optical geometry does not change. The overall process has been termed Self-Backing Reflectance Transformation (SBRT) (Matta, 1996). Data can also be processed with basic equations and CIE tables and graphs (Mark and Murakama, 1983).

Our objective was to determine the influence of sucrose replacement by glucose on color of concentrated milk plus sucrose at different moisture content, evaluating the color difference that occurs on heating.

## MATERIAL & METHODS

### Experimental design

A second-order central composite design was selected (Gacula and Singh, 1984). The variables, % of anhydrous glucose, in the glucose plus sucrose mixture (X1) and product moisture (X2), were coded between -1.41 and 1.41 according to the design (Table 1). Dominant Wave Length (D.W.L.), Excitation Purity (E.P.), and Kubelka-Munk Index  $K/S$ , were obtained from colorimetric measurements.

### Sample preparation

The basic composition of the model system was: whole dry milk powder 24g, commercial sucrose (Ingenio Ledesma S.A., Jujuy, Ar-

**Table 1—Experimental design**

Run	Sucrose replacement (X <sub>1</sub> )		Moisture (X <sub>2</sub> )	
	Coded	Uncoded%	Coded	Uncoded%
1	-1	15	-1	20
2	1	85	-1	20
3	-1	15	1	35
4	1	85	1	35
5	0	50	0	27.5
6	0	50	0	27.5
7	-1.41	0.5	0	27.5
8	1.41	99.5	0	27.5
9	0	50	-1.41	16.89
10	0	50	1.41	38.11
11	0	50	0	27.5
12	0	50	0	27.5

gentina) plus anhydrous glucose 50g, distilled water as needed to fit the experimental design (Table 1), anhydrous glucose (Mallinckrodt, USA) amount according to design (Table 1). The proximate composition (%) of milk powder Nido (Nestlé Argentina S.A., Buenos Aires) was: protein 24.95, milk fat 26.50, lactose 39.50, minerals 6.12, and moisture 2.93. Solids were dissolved in distilled water at 40°C for 30 min, with magnetic stirring. The pH was adjusted to 7.00(0.02 by addition of Ca(OH)<sub>2</sub> reagent grade (Mallinckrodt, USA) using an E516 pH meter (Metrom Herisau, Switzerland). Three mixtures were prepared for each experiment; exactly weighed samples (ca 3g each) of this mixture were placed into Pyrex glass tubes (140 mm long, 7 mm dia, 1 mm wall thickness) and sealed using a flame. Two sample tubes were taken from each preparation and subjected to heat treatment for 18 min at 115±0.1°C in a silicon oil bath (Haake, Karlsruhe, Germany) and then cooled to room temperature.

### Optical measurements

Nine very different papers and three kinds of dulce de leche were tested to select the reference sample. One of the bright, high-scattering papers, with a relative difference between standard R<sub>∞</sub> measurement and SBRT ≈0.5%, was selected due to its better values and ease of use. When measuring R<sub>∞</sub> of the reference samples, the standard procedure was followed (USA TAPPI Test Methods, 1996/97). From previous trials, a few cylindrical containers (28 mm dia and 4.5 mm depth) were adopted for all SBRT measurements. Those used in the R<sub>x</sub> test were painted white (nonblack backing) and those for the R<sub>0</sub> test were painted black (black backing). A 0.070-mm film of Mylar was placed between the specimen and the integrating sphere, to protect the device from sample stickiness. Special care was taken to avoid entrapped air bubbles in the light path, inside the sample or between the Mylar and the sample. A DFC 5 Elrephomat spectrophotometer (Carl Zeiss, Germany) with a standard source (illuminant "A" was used, according to international standards (USA TAPPI Test Methods, 1996/97). SBRT values were calculated with an ad hoc software of the same name (Matta, 1996). The additional input of the weight of the sample enabled calculating both scattering and absorption coefficients separately. Accuracy of the SBRT method and equation set was checked against standard R<sub>∞</sub> measurement over different materials. The relative difference between standard and SBRT was always <2%, mostly around 0.1-0.5%.

### Statistical analysis

Results were subjected to regression analysis, variance analysis, and surface response analysis, using *Table Curve* (Jandel Scientific Software, 1993) and *Statistica* (Statistica, 1993) programs.

## RESULTS & DISCUSSION

WE COMPARED (TABLE 2) THE COLORIMETRIC MEASUREMENTS and calculated absorption coefficients K, scattering coefficients S and Kubelka-Munk Indexes K/S. A small standard deviation of E.P. (σ = 4.62) and an even smaller one for D.W.L. (σ = 1.23) were ob-

**Table 2—Kubelka-Munk index, absorption and scattering coefficients, and colorimetric measures**

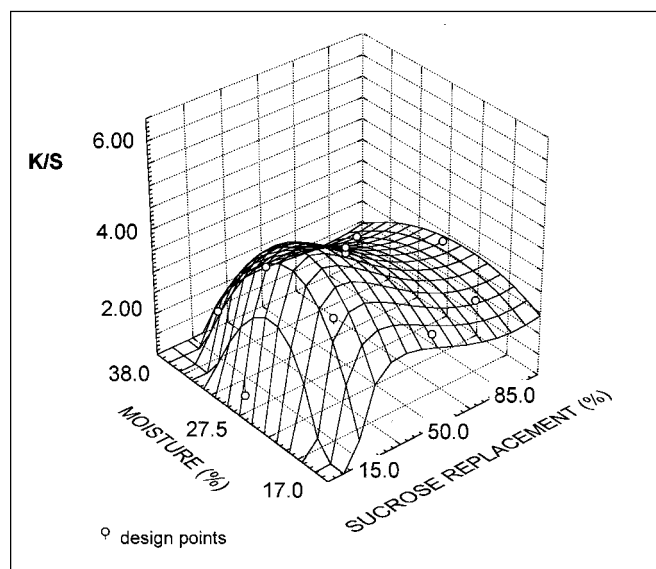
Run	K (m <sup>2</sup> kg <sup>-1</sup> )	S (m <sup>2</sup> kg <sup>-1</sup> )	K/S	E.P. (%)	D.W.L.(nm)
1	0.43076	0.11107	3.87820	58.21	583.94
2	0.46412	0.17116	2.71164	64.77	582.56
3	0.37575	0.17635	2.13068	50.85	581.18
4	0.46193	0.19117	2.41639	56.83	581.73
5	0.53344	0.14531	3.67104	60.36	582.45
6	0.61779	0.16237	3.80477	59.57	582.45
7	0.27562	0.19545	1.41023	50.57	578.96
8	0.46176	0.15924	2.89980	61.07	581.95
9	0.56058	0.18049	3.10574	65.88	583.35
10	0.44944	0.21895	2.05274	56.96	581.69
11	0.55485	0.15324	3.62079	59.80	582.46
12	0.55485	0.14996	3.78087	60.00	582.45

served despite the color differences perceived between samples, indicating that these parameters did not explain the color differences perceived by consumers. On the contrary, the Kubelka-Munk Index has been reported as a good indicator of the visually perceived color differences in products such as dulce de leche (Buera et al., 1990). Data showed good fit with the following mathematical model:

$$K/S = (3.73804 + 1.08007X_1 - 0.49172X_1^2 + 0.35945X_1^3 - 0.16176X_2 - 0.61290X_2^2) / (1 + 0.58766X_1 + 0.06468X_2)$$

$$r^2 = 0.994; \text{ Std. Err.} = 0.12528$$

This model explained 99.4% of the variability of K/S with a nonsignificant lack of fit (α = 0.358). The corresponding contour lines showed that color becomes darker (greater K/S) as total solids increased, except at high glucose content (85% of sucrose replacement) for which K/S remained quite constant as moisture varied (Fig. 2). For a given moisture content, increasing sucrose replacement by glucose led to a maximum browning zone with K/S value depending on moisture. From this zone, the tendency changed slightly and color became less dark as % of glucose in the sugar mixture increased. This darkest zone became narrower and darker between 20 and 30% of moisture content. The product reached smaller K/S values at high moisture content and above 15% of sucrose replacement by glucose, showed little sensitivity to glucose changes. Apparently, sugar concentration would be the main factor limiting browning reaction rate in this case. Little sensitivity of K/S to glucose changes was also observed when moisture content was <20%,

**Fig. 1—Surface plot of Kubelka-Munk Index (K/S) by variables: moisture content and sucrose replacement by glucose.**

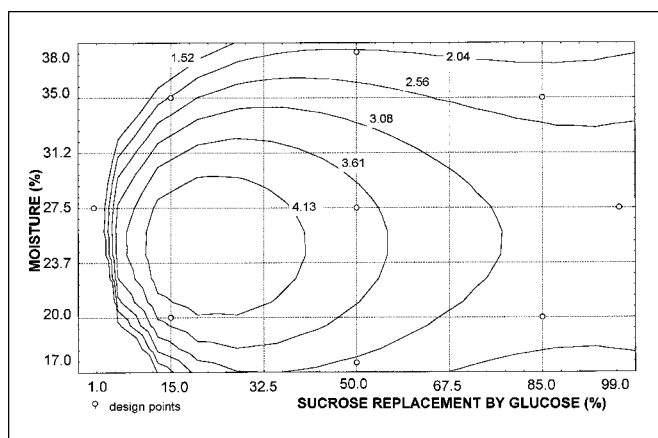


Fig. 2—Contour lines of Kubelka-Munk Index (K/S) by variables: moisture content and sucrose replacement by glucose.

although a little more browning resulted. In this case, the lower  $a_w$  would possibly be a limiting factor in browning rate. This confirmed several reports on the noticeable influence of  $a_w$  on the browning rate of dairy products, especially condensed milk (Walstra and Jenness, 1987). According to the Maillard reaction mechanism, glucose reacts faster than sucrose, or generally other disaccharides, with protein amino groups (Buera et al., 1987).

Results have shown that above a certain sucrose replacement by glucose, additional sucrose replacement did not increase browning (Fig. 2). Also, it can produce the opposite effect, decreasing browning depending on moisture content. This supports the findings that color development in this type of system is due not only to Maillard chemical browning but also to caramelization and, possibly, oxidation reactions (Buera et al., 1987; Pauletti et al., 1995). Probably the increase in glucose (to the detriment of sucrose) renders the sucrose concentration the limiting factor in the rate of browning, because of greater sucrose contribution to caramelization reaction rates. Consequently, the glucose increase would lead, in this case, to a smaller total browning level for a given reaction time and temperature.

The absorption coefficient K indicates the incident light proportion that was absorbed by the sample (Calvo, 1993) and is related to the number of chromophorous groups present. Response surface adequately interpreted the sucrose replacement and moisture influence on K. The heat treatment led to a zone of greater K as variables approached the design center (40-75% glucose and 20-30% moisture) (Fig. 3), indicating greater chromophorous group generation. Beyond that zone, K proportionally decreased no matter what the variable modification

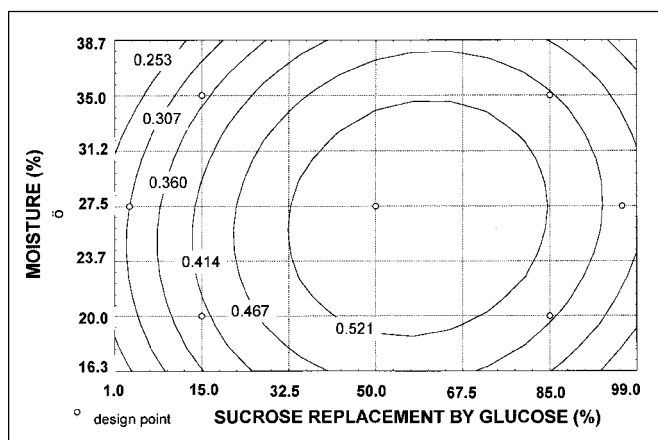


Fig. 3—Contour lines of absorption coefficient (K) by variables: moisture content and sucrose replacement by glucose.

(either increase or decrease of sucrose replacement or moisture content). The regression equation was:

$$K = 0.567 + 0.047X_1 - 0.014X_2 - 0.095X_1^2 - 0.046X_2^2 + 0.013X_1X_2$$

( $r^2 = 0.921$ ; Fit std error = 0.036)

The model proved very suitable, with a nonsignificant lack of fit. Only linear and square sucrose replacement effects were significant, according to variance analysis.

## CONCLUSIONS

THE SBRT PROCEDURE WAS VERY SUITABLE TO EVALUATE THE self-backing reflectance in small samples. Contrary to convention, at least by dulce de leche producers, results revealed two remarkable aspects: first, the increase of sucrose replacement by glucose not always led to a darker product, depending on other factors such as moisture content. Second, at high glucose content, the darkness of the product was not increased as total solids increased, probably due to a decrease in chromophorous group formation occurring when glucose replacement reached up to 85%. The Kubelka-Munk Index is an appropriate method for quality control of darkened products like dulce de leche, in which sucrose replacement by other sugars, like commercial corn syrup with different glucose contents, can be used by processors advantageously.

## NOMENCLATURE

- K Light absorption coefficient ( $m^2 kg^{-1}$ )
- $R_b$  Reflectance coefficient of nonblack backing.
- $R_0$  Reflectance coefficient of the sample over black-backing.
- $R_x$  Reflectance coefficient of the sample over backing  $R_b$ .
- S Light scattering coefficient ( $m^2 kg^{-1}$ ).
- t Intrinsic transmittance coefficient of the sample.

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