Digital image analysis and visual evaluation of orange juice: influence of different measurements conditions

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Running tittle: Digital image analysis in Orange juice

Abstract

This study assesses the effect of background, surrounding, thickness and dilutions on the colour specifications of orange juice analyzed by digital image and visual evaluation. A commercial orange juice was diluted with distilled water to give ten levels of concentration (100, 90, 80, 70, 60, 50, 40, 30, 20, and 10% of juice). These samples were evaluated instrumentally by digital image analysis using nine combinations of surroundings and backgrounds (black, grey and white) and three different thicknesses. Visual evaluation was also carried out on black, grey and white backgrounds. A grey surrounding, a white background and a fixed thickness are suggested to be used in future measurements of orange juice colour by digital image analysis. Results of visual evaluation indicated that panellists were able to order correctly the orange juice samples according to the dilution level, independently of the background chosen. The correlations between the instrumental and the visual analysis were significant *($p<0.05$)* in all the backgrounds explored.

Key words: Colour, image analysis, background, surrounding, thickness.

1. Introduction

Colour perception is a psychophysical phenomenon that involves the psychology of the observer, the physiology of vision, and the spectral radiant energy of a source of light. The visible radiation produces an stimulus on the retina, which is transmitted to the brain by the optical nerve[1]. Colour measurement is necessary in routine quality assessment and in research and development in the food industry. It is one of the most important visual attributes in food and is usually the first one evaluated by consumers. Furthermore, it is associated to the concept of quality and some studies have revealed that the colour of citrus beverages is related to the consumer's perception of flavour, sweetness and other quality characteristics[2,3,4,5].

Colour can be evaluated by instrumental and visual analysis[6]. Methods for visual assessment of colour includes preference tests, difference tests, acceptance tests[7], and descriptive analysis methods[8,9]. For this kind of evaluation, the observing conditions such as illumination, sample presentation, background or surrounding on which the sample is presented have to be taken into account[10].

On the other hand, instruments are able to define colour objectively following the recommendations established by theCIE[11,12].

The International Commission on Illumination (Commission Internationale de l'Eclairage, CIE) developed the Tristimulus Colorimetry, which is a valuable resource for solving the problems of objective analysis of colour in foods and beverages, thus, diverse applications have been carried out with orange juice (OJ)[13,14]. In particular, the CIELAB system has been accepted worldwide in most industries[15], starting from the tristimulus values previously defined by CIE colorimetry[16].

The main advantages of the instrumental measurement are its simplicity, precision, and versatility. They also eliminate subjectivity, are non destructive and allowautomatization[17]. There are different types of colour measurement instruments like colorimeters, spectrophotometers and spectroradiometers. Their validity for the objective colour evaluation of food and beverages, among them, orange juice (OJ), have been demonstrated by several authors[18,19].

New advances in image acquisition technology offer the possibility of using technically sophisticated apparatus available at relatively low cost to evaluate colour in terms of millions of pixels. In comparison with the traditional light sensors, the main advantage, is that they allow to make a detailed evaluation of a wider area of any food product, with inhomogeneous colour possible, and every different colour can be accounted for by one or more pixels[20]. Furthermore, it is based upon digital cameras, which can quickly capture images in digital format (DigiEye®)[21] and offers a more reliable measurement of the food colour, which can be correlated with sensory analysis[22].

As in any other instrumental measurements, it is necessary to set up the right measurement conditions considering the characteristics of the product to be measured. When the incident light goes through any translucent beverages, part of the light is reflected, part is transmitted, part is absorbed, and also part comes from the background and goes through the sample towards the observer's eye or the instrument, so accurate colour specifications in OJ is not an easy task . When measuring colour, the following aspects must be considered: the arrangement of the sample, the geometry of the system, the light source intensity, the thickness of the sample, and the blank; as they may affect the instrumental measurement[25,24.] Other authors have also suggested that background and surrounding area are factors that can also influence colour measurement[17]. Also, it is necessary to control the lighting geometry[24] which is the relative position of the sample, and the entire trajectory of the light until it reaches the observer's eye orthe instrument.

As digital image is a very accurate measure of the chromatic characteristics of each pixel, the influence of these factors becomes especially important. Furthermore, this kind of measure simulates in a very exact way the visual appreciation, in which it is clear that they have a relevant effect[10].

Therefore, the main objective of this study was to set up the right conditions for colour evaluation of orange juice by Digital image analysis. Several measurements conditions were assessed including backgrounds, surroundings, sample thickness, and dilutions. Colour specifications of OJs were analyzed by digital image and visual evaluation. Furthermore correlations between visual and instrumental analysis with different backgrounds were also explored.

2. Material and methods

2.1. Samples

A series of dilutions of commercial fresh OJ with distilled water were done to get ten levels of OJ concentrations (100, 90, 80, 70, 60, 50, 40, 30, 20, and 10% of juice).

As a result of the successive dilutions, a collection of orange juice solutions with different amount of pulp and pigments were obtained, which represent the range of OJs colours from the darkest to the palest.

2.2. Colour measurement: Image analysis

The DigiEye imaging system was used to capture the digital images[21]. The latter system includes a calibrated digital camera with 10.2-megapixel Nikon D80 (Nikon Corporation, Tokyo, Japan) and an objective Nikkor 35-mm f/2D (Nikon Corporation), a colour sensor for display calibration, and an illumination box designed by VeriVide Ltd. (Leicester, UK) (Figure 1).

To explore the influence of the surroundings and backgrounds on the colour measurement of OJ by image analysis, three backgrounds and surroundings (white, grey (L* = 50), and black) were selected. Figure 2 shows the nine combinations of backgrounds and surroundings used. The influence of the sample's thickness was also evaluated. Three levels of thickness: 15, 30, and 40 mm were obtained by filling a cuvette with 44.7 mm of internal diameter with three different volumes (20, 45 and 70 mL).

In these measurements, the samples were illuminated by a diffuse D65 simulator. To calculate the CIELAB coordinates from RGB colour space, the DigiFood software was used [26].

For colour specifications the CIELAB colour space, recommended by the CIE[27] was applied. Colour differences can be calculated by different formulas like CIE94[28] or CIEDE2000[29,30]. In this work we have decided to use the most usual CIELAB formula in OJ which calculate colour differences as the Euclidean distance between two points in the 3-D space defined by L*, a* and b*:

$$\Delta E^*_{ab} = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$$

2.3. Visual analysis

To control the illumination and observation conditions visual analyses were carried out using a VeriVide CAC Portable cabinet (Konika Minolta, Nieuwegein, Netherlands) equipped with a daylight source simulating the D65 illuminant (dimension of viewing area: 635 mm width, 280 mm height, and 280 mm depth). The visual analyses were done under the following conditions: grey colour (L* = 50) surrounding and three different backgrounds (black, grey and white) (Figure 8). To standardize thickness to 30 mm (a medium value from those assayed in the instrumental measures) the cylindrical transparent glass flasks were filled with 80 ml of the corresponding solutions. Samples were placed randomly in the cabin.

Ten panellists recruited from students and staff at the University of Seville, without previous training in visual evaluation, took part in the study. All of them were asked to order the samples in an ever-increasing order of concentration of OJ. For data analysis, the samples were given from 1to10 points, corresponding to the most diluted OJ sample to the original OJ, respectively.

3. Results

3.1. Influence of surrounding and background on the orange juice colour parameters assessed by digital image.

In order to explore the effect of the background and the surrounding on the colour analysis, an average diluted sample (50%) was selected. Thickness was fixed at 30 mm. The influence of the surrounding was evaluated by comparing the colour differences for white/black (SW/SB), white/grey (SW/SG), and black/grey (SB/SG) surroundings with all the backgrounds explored.

The highest colour differences (ΔE^*_{ab}) were observed when comparing SW/SB, in which colour differences ranged from 6.84 to 6.87 for the three different backgrounds. The second highest colour differences corresponded to SW/SG (4.70-5.08). In both cases, ΔE^*_{ab} was higher than the visual discrimination threshold which has been established for wine by Melgosa et al.[31] (ΔE^*_{ab}>3 CIELAB units approximately). The lowest colour differences, ranging from 1.85 (white background) to 2.35 (grey background), were obtained when comparing the surroundings SB/SG.

These results are in agreement with Stinco et al.[17], who conducted a similar study on spectroradiometric instrumental measurements. They concluded that the highest colour differences were obtained when comparing SW and SB, although they reported higher ΔE^*_{ab} values.

Although many studies have reported the influence of different backgrounds on the instrumental measurement of colour[18,32,6,25,17] none of them have been referred to digital image analysis. To explore the influence of the background, the average diluted sample (50% of OJ) was also selected and three possible backgrounds: white background (BaW), grey background (BaG), and black background (BaB). Colour differences due to the background were calculated. The highest colour differences (ΔE^*_{ab}) were observed for the pair BaW/BaB, ranging from 2.72 to 2.99 for the three different surroundings. The second highest colour differences corresponded to BaG/BaW (2.30-1.75), and finally the lowest colour differences were obtained when comparing the backgrounds BaB/BaG, ranging from 0.72 (SW) to 1.00 (SG). All these differences were lower than the threshold of visual detection reported by Martínez et al.[33]. It must also be highlighted that the backgrounds produced lower colour differences than the surroundings, as reported above for the 50% OJ sample. Similar results were reported by Stinco et al.[17] in spectroradiometry measurements, although the colour differences reported in that study were higher (2 - 18 CIELAB units). Similarly, other authors have also concluded that background has a clear effect on the instrumental colour measurements of OJ[10] and meat[34].

Figure 3 shows the location of the 50% OJ sample in the *a**b** plane as a function of the background used. It can be observed a clearly separated group corresponding to BaW and two other closer groups corresponding to BaG and BaB, which showed lower values of a* and b* (Figure 3a). However, Figure 3b shows that when representing L* values, no well-defined groups are observed. Obviously, the lowest values of L* corresponded to the black background (BaB) while the highest values of L* to the white background (BaW). However, the fact that the samples showed no grouping tendency, suggests that the effect of the background in OJ does not have a relevant impact in lightness values.

The influence of the surrounding and background on the colour parameters is affected by the dilution of the sample, as it can be inferred from the collection of different dilutions of OJ samples. Figure 4 shows that, when comparing surroundings, the colour differences decreased from the most diluted OJ (10%) to the most concentrated (100%). The same trend was observed for the different backgrounds. For example, OJ 10%, on BaW showed colour differences values of 10.45, 7.09, and 4.19 on SB/SW, SG/SW, and SB/SG, respectively, while for OJ 100%, colour differences values decreased to 4.78, 3.62, and 1.24, respectively. This implies a reduction around 50% in colour differences values.

Figure 5 shows the effect of the backgrounds for the ten dilutions studied. As previously discussed for the surroundings, the same trend was observed when comparing the different backgrounds on the three surroundings. Furthermore, the differences in colour comparing backgrounds also decreased due to OJ concentration, but in a more accused way. Taking as an example the sample OJ 10% in SW, the colour differences were 11.38, 7.81, and 3.82 comparing BaW/BaB, BaG/BaW, and BaB/BaG, respectively, while for OJ 100% colour differences values decreased to 0.99, 0.41, and 0.37, respectively. In this case, a reduction around 90% in colour differences values was observed due to the effect of the background.

It can be concluded that surrounding as well as background do have an influence in the digital analysis measurements of OJ. The magnitude of this effect is affected by the concentration of pulp and pigments. Due to the huge offer of OJ existing in the market, with different amount of pulp and pigments, it is necessary to fix these factors before OJ colour analysis. Therefore, according to these results and taking into account the CIE recommendations[27] and also previous studies with different instrumental measurements[17], the grey surrounding (uniform neutral grey with L^* = 50) and white background were selected for the following measurements. Besides the CIE recommendation cited above, this combination is also the most usual in visual assessment.

3.2. Influence of dilutions and thickness on the orange juice colour parameters assessed by digital image.

The effect of the dilutions on the colorimetric parameters was also studied in this work. It can be observed that the lower the concentration of the OJ, the higher the values of L* (Figure 6a). Hue was affected in a similar way: the lower the concentration of the OJ, the higher values of h_{ab}. This means that OJs with more pigments in pulp and serum are more reddish and darker (as it could be expected) (Figure 6c). The same trend was observed by Stinco et al.[17] and Meléndez Martínez et al.[10]. Finally, in the case of chroma, it can be observed a slight increase for OJ dilutions ranging from 10 to 40 %, and a stabilization of C^*_{ab} values from this dilution onwards (Figure 6b). However, other authors Melendez-Martínez et al.[10] and Stinco et al.[17] have reported that C^*_{ab} values increases as the concentration of OJ does.

The effect of the sample thickness on the chromatic parameters (L*, C^*_{ab}, h_{ab}) is shown in Figure 7. The colorimetric parameter lightness decreased as the thickness increased, however this effect was less important as the concentration of OJ increased. This means, that the effect of thickness on L* is negligibly when the concentration of the OJ is 100% (Figure 7a). The same trend is observed for chroma. For samples containing over 40% of OJ, the effect of thickness is null (Figure 7b). Hue is also represented in Figure 7c. This parameter was not affected by the sample thickness.

Previous studies have been conducted on the effect of thickness in colour measurements but in other foods and beverages[35,24]. For example, studies in olive oil found out that lightness and hue decreased when increasing sample thickness while chroma increased up to about 19.6 mm thickness. They also concluded that colour tended to stabilize at a certain thickness[24].

It could be concluded that the effect of the thickness will become lower as the pulp and pigments content increases because of the decline of the OJ translucency. However, the thickness must be taken into account and fixed, in order to do reproducible and reliable objective colour measurements by digital image.

3.3. Visual analysis

In order to compare the instrumental and the visual analyses, 10 panellists took part in the visual assessment of the different samples considered. Each of them was asked to sort the series of cuvettes containing the different dilutions of orange juice in increasing order of OJ concentration. Table 1 shows the rank sums (RS) given by the panellists in the different backgrounds. The inter-variability of the panellists has been calculated considering the correct responses of each one, obtaining an average punctuation of 7.2 ± 1.4.

On black and white backgrounds two completely correct visual assessments were achieved, while on grey background one more was achieved. It has to be highlighted that in the incorrect assessments, the main mistakes were done among samples with more than 50 % of OJ. Thus, if only the most diluted samples were taken into account (10, 20, 30, 40, and 50 %) the percentage of completely correct visual analyses would increase up to 80 - 90% of the panellists. This means that it is more difficult to discriminate among samples with higher pulp and pigment contents. These results could be explained by the colour differences between consecutive samples, measured on the different backgrounds. Table 2 shows that from the pair 40-50 % and 50-60 % to the pair 90-100 %, colour differences decreased considerably.

In any case, the statistical analysis by the Page test of the set of data, showed that all of the backgrounds resulted in significant correct classifications *($p<0.05$)* and all the backgrounds showed a similar pattern.

The similarity in the ordering results obtained for the different backgrounds, could be explained by the previous instrumental results discussed above. They showed that colour differences between backgrounds were lower than the colour-difference threshold reported by the literature in many of the samples and furthermore, the same trend was observed in all the backgrounds (Figure 4 and 5). In contrast to these results, previous studies on the effect of the background on the visual evaluation of OJ showed that the best results were obtained when a cabin with black walls was used[10]. Huang et al.[36] also concluded that panellists were able to rank squash purees samples with the highest correlations with the theoretical order, when a black background and was used.

3.4. Correlation between visual and instrumental measurements

The correlations between the instrumental and the visual data were explored by means of a polynomial regression. The RS given by the panellists (Table 1) and each of the colorimetric coordinates (L*, C^*_{ab}, and h_{ab}) in the different backgrounds were considered. Results are presented in Table 3. Chroma did not present a good adjustment with any of the backgrounds explored (R^2 values were 0.61, 0.55 and 0.56 with BaB, BaG and BaW, respectively). Hue was very well correlated with RS in all the backgrounds (R^2= 0.99), and in the case of L*, the best correlation was obtained for white background.

Multiple regressions analysis between instrumental and visual data, considering the RS given by the panellists (Table 2), was also explored, and all the correlations obtained were significant *($p<0.05$)*:

Black background:

$RS = -22.553 + 1.235L^* + 7.786a^* - 0.296b^*$; $R^2=0.993$

$RS = 909.09 -0.519L^* - 1.406C^*_{ab} - 8.310h_{ab}$; $R^2=0.990$

Grey background:

$RS = - 570.84 + 10.339L^* + 10.999a^* - 1.608b^*$; $R^2=0.990$

$RS = 655.21 + 10.317L^* - 3.659C^*_{ab} – 12.260h_{ab}$; $R^2=0.997$

White background:

$RS = 194.94- 2.181L^* + 7.134a^* - 0.120b^*$; $R^2=0.984$

$RS = 681.70 + 7.883L^* - 0.753C^*_{ab} - 12.864h_{ab}$; $R^2=0.988$

4. Conclusions

In conclusion, the results of this work have probed the influence of the surrounding and the background in the definition of OJ colour by image analysis. According to them and considering the CIE recommendations and other previous studies, the grey surrounding (uniform neutral grey with L* = 50) and white background are proposed to be used in the colour determination by digital image analysis of OJ. Moreover the sample's thickness must be also taken into account and fixed to get reproducible and reliable colour measurements of OJ by digital image.

In the visual evaluation of OJ the background is not relevant, thus the correlations between the instrumental and the visual analysis were significant *($p<0.05$)* in all the backgrounds explored.

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Compliance with Ethics Requirements

Rocío Fernández Vázquez holds a grant from the Consejería de Innovación Ciencia y Empresa, Junta de Andalucía.

Carla M. Stinco declares that she has no conflict of interests.

Dolores Hernanz declares that she has no conflict of interest.

Francisco J. Heredia, declares that he has no conflict of interest.

Isabel M. Vicario declares that she has no conflict of interest.

This article does not contain any studies with human or animal subjects.

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Figure Captions

Figure 1. Scheme of DigiEye System.

Figure 2. The nine possibilities of combinations of backgrounds (black (BaB), grey (BaG) and white (BaW)) and surroundings (black (SB), grey (SG) and white (SW)) used in the digital image analysis.

Figure 3. Location, of the sample (OJ 50%) measured in the different background and surroundings, in the diagram: (a) in the a*b* plane and (b) in L* values.

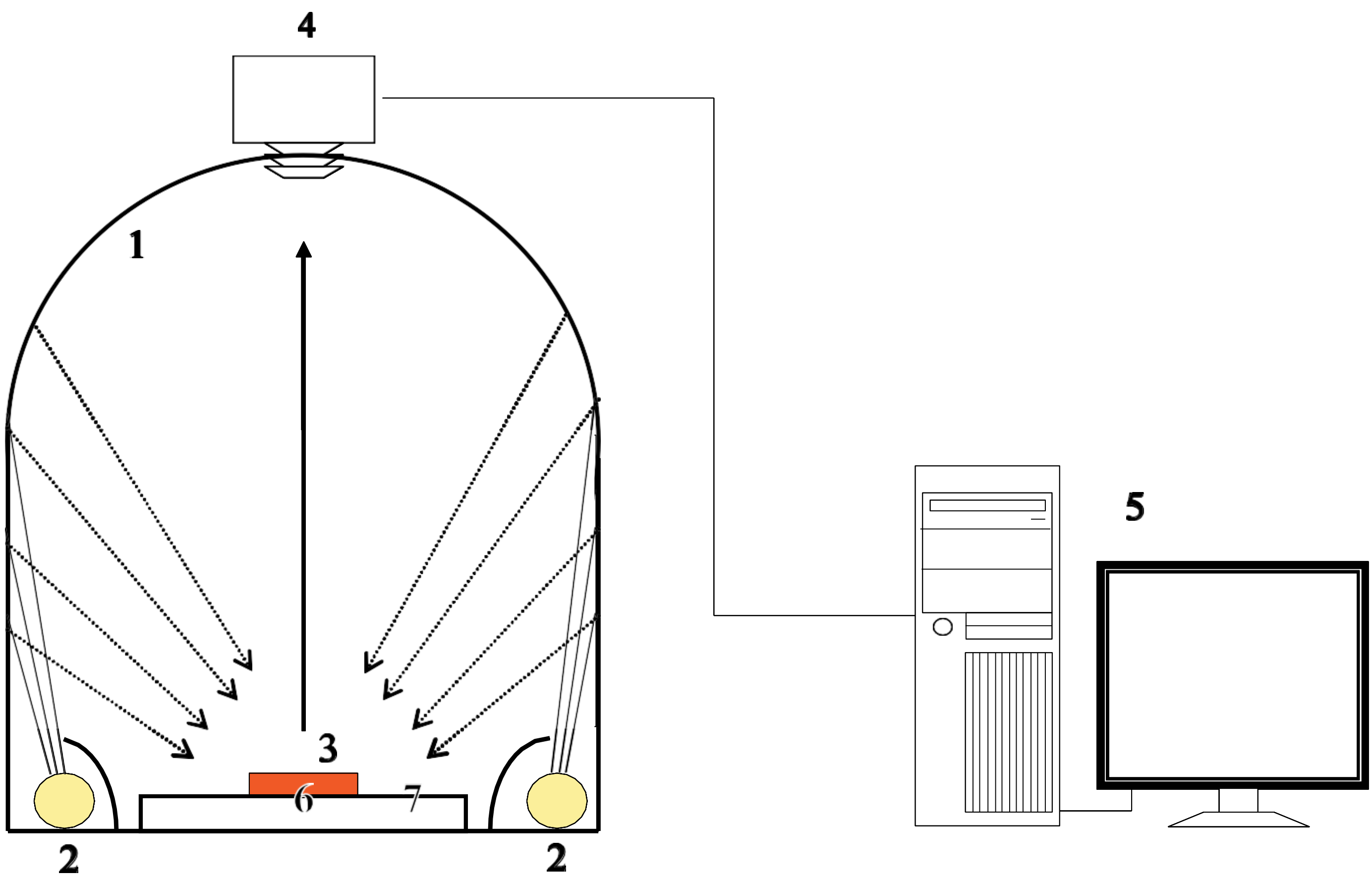
Figure 4. Colour differences between surroundings (SB/SG, SB/SW, and SG/SW) with black (a), grey (b) and white (c) backgrounds.

Figure 5. Colour differences between backgrounds (BaB/BaG, BaB/BaW, and BaG/BaW) with black (a), grey (b) and white (c) surroundings.

Figure 6. Effect of the orange juice (OJ) dilutions in the colorimetric parameters.

Figure 7. Effect of orange juice (OJ) samples' thickness on L*, C^*_{ab} and h_{ab}.

Figure 8. Pictures of the three viewing conditions (BaB, BaG, BaW corresponding to black, grey and white background)



1: Domed cabinet
2: Fluorescent tubes (D65 simulator)
3: Sample
4: Digital camera
5: PC with Digifood® Software
6: Background
7: Surrounding

Figure 2
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Figure
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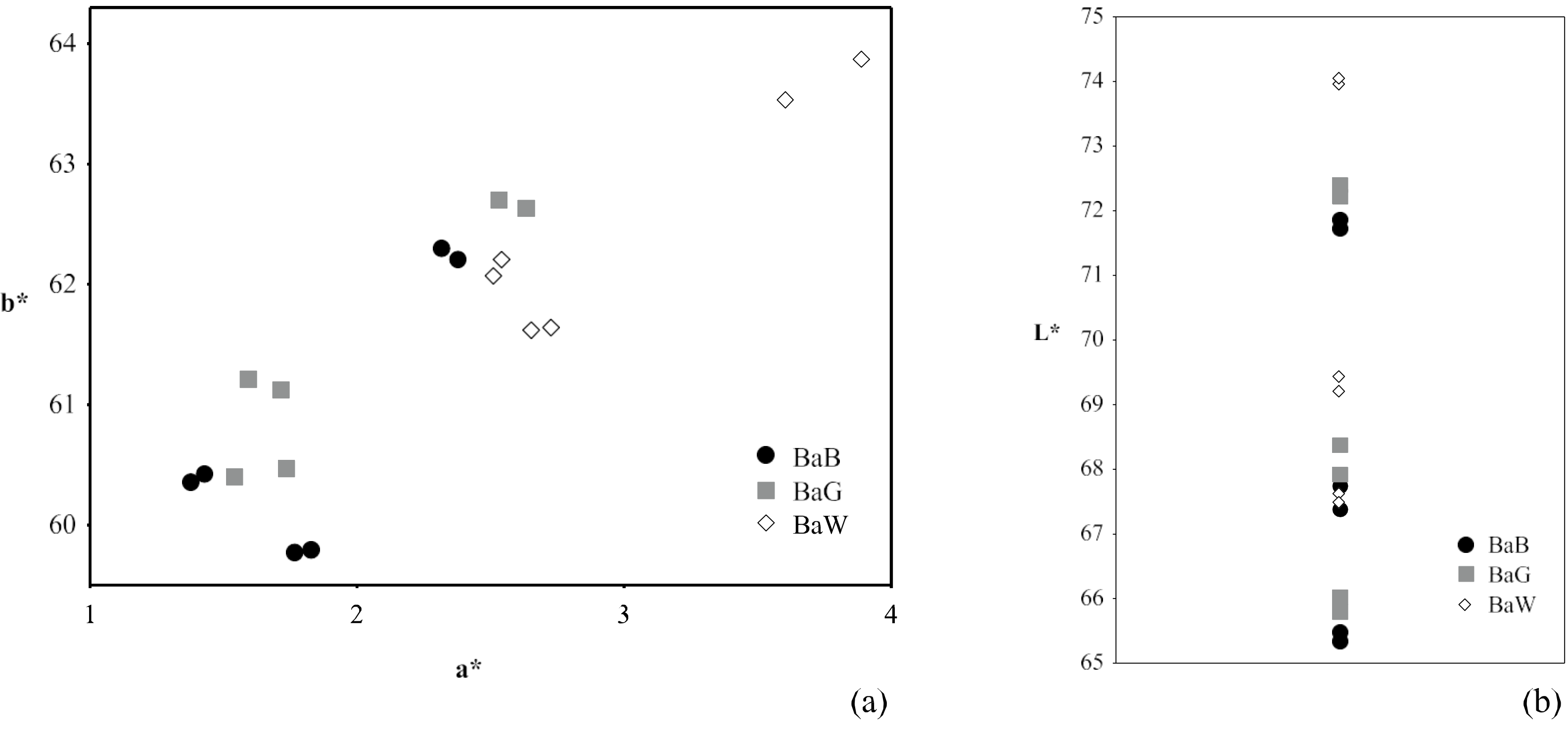

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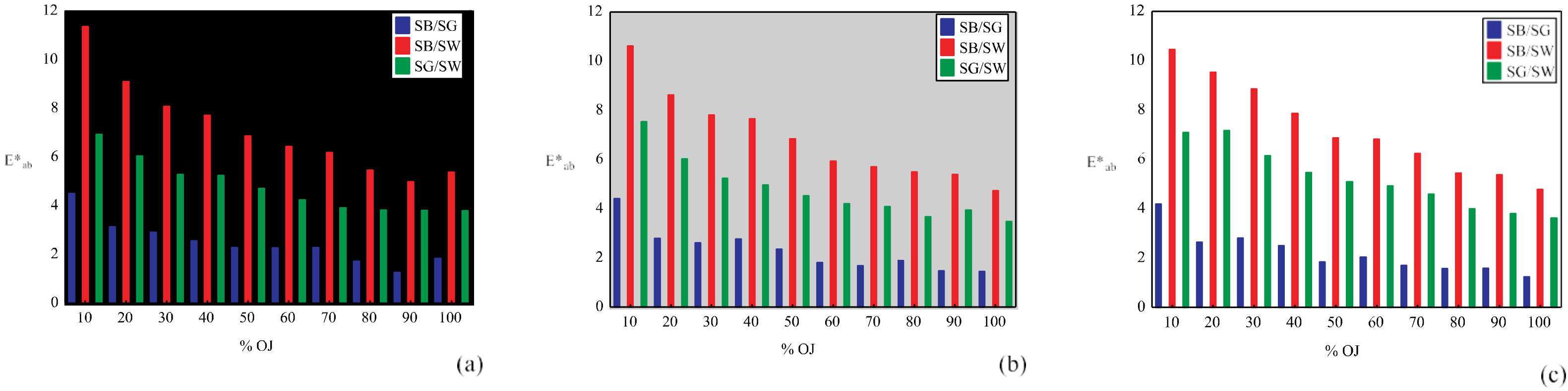

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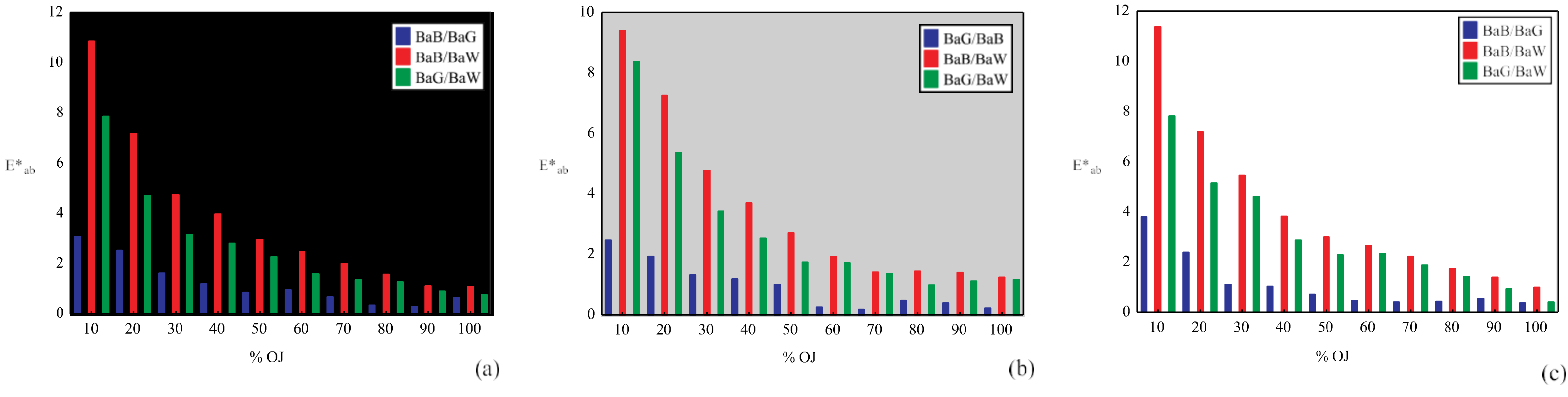

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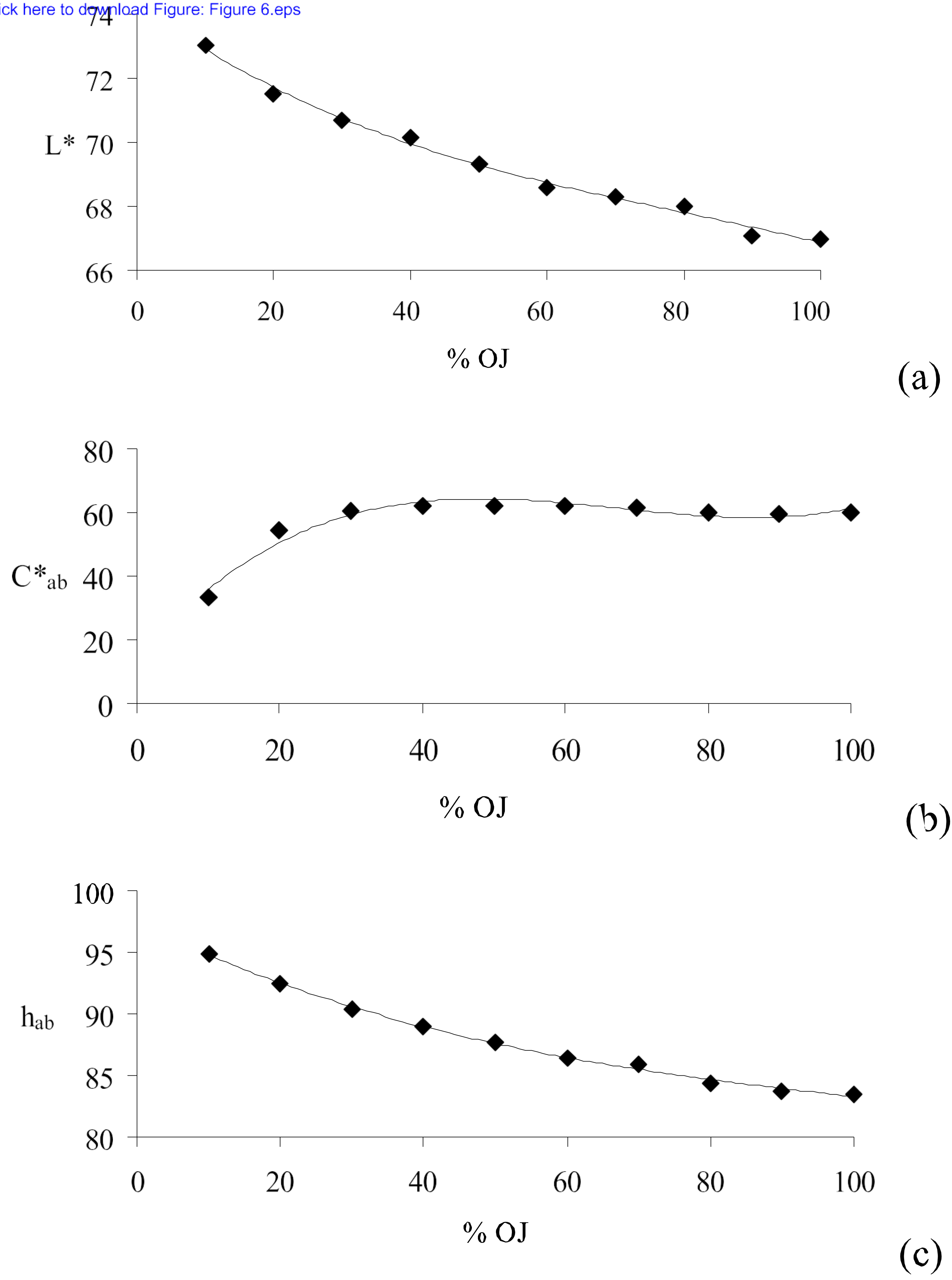

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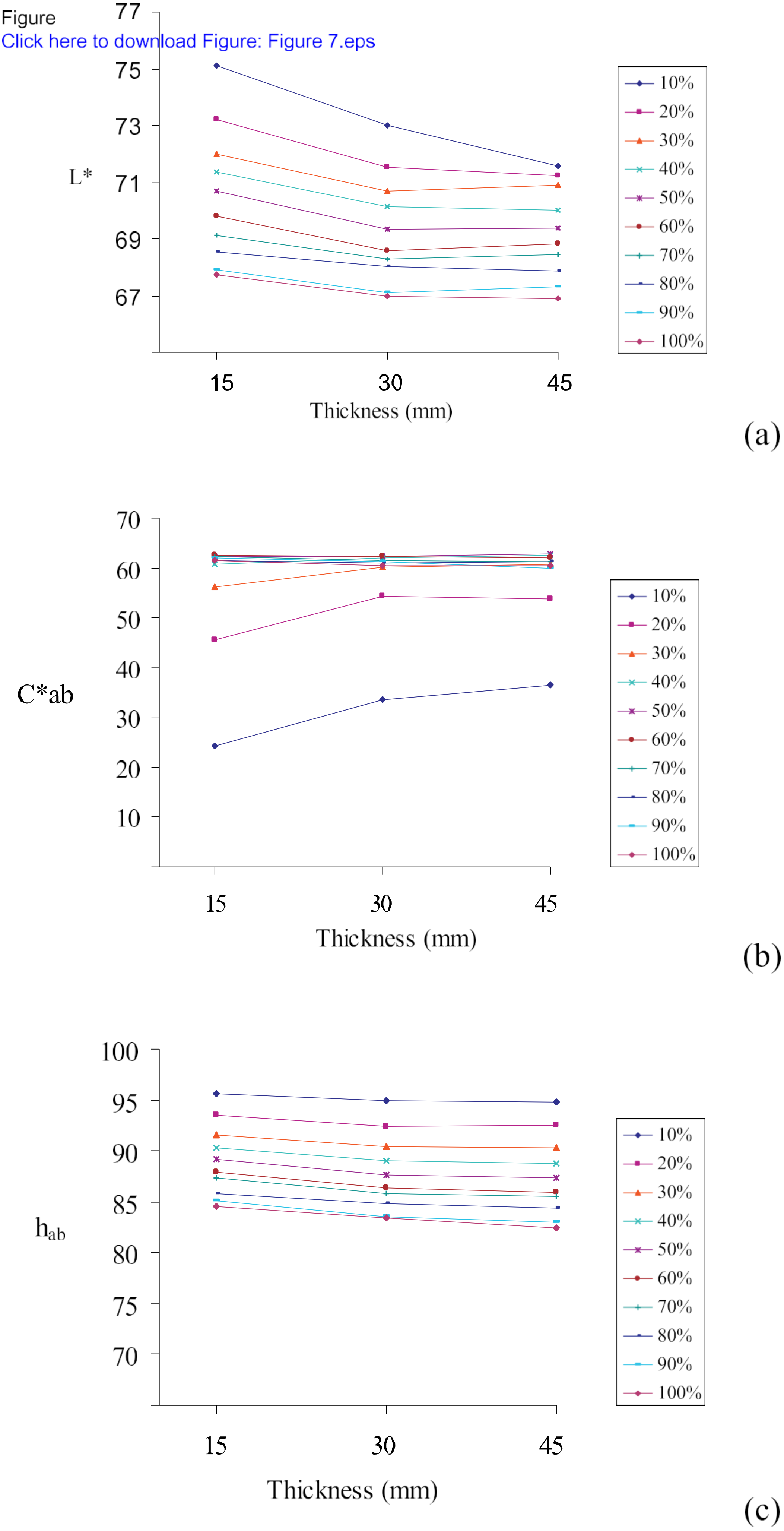

Figure 8

Table 1. Rank sums (RS)of the panellists in black (BaB), grey (BaG) and white (BaW) backgrounds.

% PULP	RS-BaB	RS-BaG	RS-BaW
10	10	10	10
20	20	20	20
30	31	30	30
40	39	41	41
50	51	49	49
60	70	63	68
70	71	70	66
80	80	85	86
90	88	86	91
100	90	96	89

Table 2. Colour differences between dilutions in black (BaB), grey (BaG) and white (BaW) backgrounds.

ΔE^*_{ab}	BaW	BaG	BaB
10-20 %	21.00	21.29	19.30
20-30 %	6.25	7.75	8.09
30-40 %	2.39	2.94	3.22
40-50 %	1.69	1.83	1.77
50-60 %	1.55	1.67	1.69
60-70 %	0.97	0.70	0.80
70-80 %	1.30	1.34	1.43
80-90 %	1.57	1.54	1.56
90-100 %	0.88	1.32	1.40

Table 3.Regression coefficients between colour parameters and rank sums (RS) of the panellists.

	BaB	BaG	BaW
L*	0.53	0.88	0.98
C^*_{ab}	0.61	0.55	0.56
h_{ab}	0.99	0.99	0.99