Scientific Journal of Gdynia Maritime University
Zeszyty Naukowe Akademii Morskiej w Gdyni

Nr 106/2018, 29–40
ISSN 2451-2486 (online)
ISSN 1644-1818 (printed)
DOI: 10.26408/106.03

Złożony/submitted: 11.06.2018
Zaakceptowany/accepted: 17.10.2018
Opublikowany/published: 18.12.2018

ATTEMPT OF ROOIBOS AND HONEYBUSH IDENTIFICATION BASED ON THE BEVERAGES COLOR PARAMETERS

PRÓBA IDENTYFIKACJI ROOIBOS I HONEYBUSH NA PODSTAWIE PARAMETRÓW BARWY NAPARÓW

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Abstract: Rooibos (*Aspalathus linearis)* and honeybush tea (*Cyclopia species),* are the products coming from South Africa, that due to their organoleptic features and antioxidant properties are gaining increasing popularity among the Polish consumers. Due to the high similarity, these products are very often confused, and both consumers and sellers have problems in distinguishing them. Therefore, the aim of the paper is the attempt to differentiate rooibos and honeybush infusions based on the consumer evaluation of color of the beverages and color parameters measured in the CIEL*a*b* system. On the basis of the obtained results, statistically significant differences in the consumer color assessment of infusions were found. The results presented in this paper indicate that the samples of rooibos were very similar and were characterized by more intensive brick, red and brown color than honeybush. However, it was shown that the values of the color parameters measured in the CIEL*a*b* system do not allow for clear differentiation of rooibos and honeybush beverages.

Keywords: rooibos, honeybush, sensory evaluation, color parameters, identification.

Streszczenie: Rooibos (*Aspalathus linearnis*) oraz honeybush (*Cyclopia spp.*) to produkty pochodzące z Afryki Południowej, które ze względu na swoje walory organoleptyczne i właściwości przeciwutleniające zyskują coraz większą popularność wśród polskich konsumentów. Ze względu na duże podobieństwo produkty te bardzo często są mylone i zarówno konsumenci, jak i sprzedający mają problemy z ich odróżnieniem. W związku z tym celem pracy była próba różnicowania naparów rooibos i honeybush na podstawie parametrów barwy, oznaczonych organoleptycznie i instrumentalnie w systemie CIEL*a*b*. Wykorzystując uzyskane wyniki, stwierdzono statystycznie istotne różnice w konsumenckiej ocenie barwy badanych naparów. Napary rooibos oceniono jako napary o większym nasyceniu barwy ceglastej, czerwonej i brązowej niż napary honeybush. Wykazano jednak, że wartości parametrów barwy mierzone w systemie CIEL*a*b* nie pozwalają na jednoznaczne różnicowanie naparów rooibos i honeybush.

Słowa kluczowe: rooibos, honeybush, analiza organoleptyczna, parametry barwy, identyfikacja.

1. INTRODUCTION

Rooibos tea is a herbal tea product coming from the endemic South African fynbos plant, *Aspalathus linearis* and production is mainly concentrated in the Clanwilliam area, Western Cape, South Africa. The genus *Aspalathus* (*Fabaceae, Tribe Crotalarieae*) is comprised of more than 270 types of which most are endemic to the Cape Floristic Region. Rooibos tea, produced from *A. linearis* (Burm.f.) Dahlg., had no commercial value at the beginning of the 20th century, but today it is a well-known herbal tea, enjoyed in more than 37 countries, including Poland [Joubert and de Beer 2011]. Rooibos is a rich source of polyphenols and is used to make a mild-tasting tea containing no caffeine. It is low in tannins compared to green or black teas. There are two types of rooibos tea: unfermented and fermented. The unfermented product remains green in color and is referred to as green rooibos. During fermentation, the color changes from green to red, with oxidation of the constituent polyphenols, so the final product is often referred to as red tea or red bush tea. The most important characteristics of rooibos are its antioxidant and antimutagenic properties [Morton 1982; Van der Merwe et al. 2006; Villaño et al. 2010; Joubert and de Beer 2011; Kotina et al. 2012]. In terms of polyphenol composition, only *C. intermedia* (fermented) and *C. subternata* (unfermented) have been comprehensively studied. The presence of a number of polyphenols, previously identified in these two species, has been confirmed in *C. genistoides* and *C. sessiliflora*. The major compounds, present in all species analysed to date, are the xanthones, mangiferin and isomangiferin, and the flavanone, hesperidin. Other compounds identified in *Cyclopia spp*. include flavanones (hesperetin, naringenin, eriocitrin, narirutin), flavones (luteolin, scolymoside and diosmetin), isoflavones (afrormosin, calycosin, wistin, orobol, pseudobaptigenin and isosakuranetin), flavonols (several kaempferol glucosides), coumestans (medicagol and sophoracoumestan) and others (epigallocatechin gallate and p-coumaric acid). Tannins in *Cyclopia spp*. are of the proanthocyanidin type [Marnewick et al. 2005] and have been estimated at ca 4.34% of the hot water soluble solids of fermented honeybush [Ferreira et al. 1998; Kamara et al. 2003; 2004; Joubert et al. 2011].

Honeybush tea, produced from *Cyclopia species*, is one of the traditional South African herbal teas with a long history of regional use; yet, unlike rooibos (*Aspalathus linearis*), it remained a cottage industry until the mid-1990s. The name “cyclopia” is derived from the Greek word “cyclops” meaning “round-eyed”, which refers to the circleshaped depression or sunken area in the base of the calyx where the pedicel is attached to the yellow flower. This feature, together with trifoliate leaves, distinguishes all *Cyclopia spp*. from related genera. The earliest reference to honeybush is found in a European taxonomic script of 1705 [Kies 1951]. C. Thunberg, a Swedish botanist, recorded the use of the name “honigtee” (Dutch) during his travels in the Cape in the 1770s. The general names, “honigtee”, honeybush and “heuningtee” or “heuningbostee” (Afrikaans), for the *Cyclopia spp.*

derived from the sweet, honey-like scent of the plant when in full bloom. C. Latrobe, travelling in the Langkloof area in 1815, was served “tea-water”, prepared by the inhabitants from a local plant, believed to be honeybush. Traditionally the leafy shoots and flowers were fermented and dried to prepare tea. The first mention of a specific species in terms of its use as a tea was by Greenish in 1881 in a report on an anatomical and chemical study of *C. genistoides* (Cape tea, “honig-thee”). A decoction of the plant was used as a restorative and as an expectorant in chronic catarrh and pulmonary tuberculosis. Other species with a history of use as a tea were *C. vogelii*, later renamed *C. subternata*, *C. latifolia* and *C. longifolia* [Joubert et al. 2011]. Honeybush is well-known as a caffeine-free, low tannin, aromatic herbal tea with a wealth of polyphenolic compounds associated with its health-promoting properties [Joubert et al. 2008]. Honeybush infusions were reported to contain 0.59 mg/ml fluoride, although no information was given on the *Cyclopia spp*. analysed. The content of aluminium, as well as mineral nutrients, was shown to be much less than that of *Camellia sinensis teas*, except for Ca which was higher [Malik et al. 2008].

Rooibos and honeybush beverages are not only consumed for the enjoyment of their taste and aroma, but also for their medicinal properties. Rooibos and honeybush have been found to provide relief for allergies, dermatological problems, asthma, infantile colic and other gastrointestinal complaints, such as nausea and heartburn. It has also been reported that these beverages can improve appetite, reduce tension and improve sleep [Kokotkiewicz and Łuczkiewicz 2009]. Rooibos tea is not well-known by the general public for its antimicrobial activity, but there have been studies confirming the inhibitory effects of rooibos against certain microorganisms, such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans* and *Candida albicans* [Joubert et al. 2008].

The classification of food samples is of great interest for identification of the product's geographical origin and authenticity, or for establishing characteristics of products [Luykx and Van Ruth 2008]. Color of foods is the first contact point of the consumer with it, so it strongly influences consumers’ preferences. Color has a major role in the acceptability of the product and is related to the consumer perception of flavor, sweetness, scents and other physical properties in relation to the quality of the product, for example wine, especially of red ones [Pérez-Magariño and González-Sanjosé 2013]. The color provides information about defects, the type, and also has an important influence on the overall acceptability by consumers[Heras-Roger, Diaz-Romero and Darias-Martin 2016]. According to a recent survey of literature, to date no many research has been reported using, for example color parameters (CIEL*a*b*) for the determination differences between rooibos and honeybush, which is relevant considering that this analysis can be inserted into the trade market allowing important conclusions about the origin of rooibos and honeybush. Therefore, the main objective of this work is to propose a simple and cheap methodology to differentiate between samples of rooibos and honeybush based on the sensory quality (especially color) of the beverages and color parameters measured in the CIEL*a*b* system. By considering the hypothesis that some differences can occur between colors of samples from different regions of origin, models based on sensory color evaluation and CIEL*a*b* coordinates of rooibos and honeybush have been developed. It could be very useful for producers and first of all for consumers, to distinguish rooibos from honeybush.

2. MATERIAL AND METHODS

The samples of rooibos and honeybush were obtained from the Tri-city market. The samples originated from South Africa and from three producers.To the research were used respectively three brands of rooibos: Kawon (R_K), Lord Nelson (R_LN) and Samowar (R_S) and three brands of honeybush: Bio Miodokrzew NatVita (H_BM), Czas na Herbatę (H_CnH), Simon Lévelt (H_SL). All samples of rooibos and honeybush should have the same form; therefore leaves were ground in a mortar. Tea infusions were prepared with water. 100 ml of deionised water (95ºC) was added to 2.0 g of rooibos tea and brewed for 5 minutes without additional heating. All samples were filtered through Whatman No. 4 filter paper.

2.1. Consumer evaluation of the color in rooibos and honeybush samples

The samples were served at the temperature range from 70 to 60ºC. Because the temperature of the freshly brewed rooibos and honeybush was around 95ºC, the samples were cooled at room temperature to until served. A total of 3 rooibos and 3 honey samples were evaluated in two sessions on different days and all of the samples were rated twice. A group of thirty consumers (15 women, 15 men) in the age range from 18 to 47 made a semiconsumer assessment of quality of beverages from *Aspalathus Linearis* and *Cyclopia spp*. Participants were consecutively selected from volunteers on the basis of non-probability sampling technique according to the convenience sampling [Martinez-Mesa et al. 2016]. The deciding factor for the selection of participants was the frequency of consumption of rooibos and / or honeybush beverages. Consumers were each time presented with 3-digit code, suitably coded, with unit samples of infusions (50 ml). Consumers, in addition to the general desirability of the infusions under study, also evaluated the intensity of selected color descriptors, which were selected based on studies carried out by Koch et al. [2012] and Dmowski and Szczygieł [2017]. Among the parameters determined were brick, red, brown and amber, orange color and brightness of the beverages. To assess the intensity of the discriminants, a simplified, structured linear numerical-descriptive scale with 0-5 contract units was used, where 0 meant the lack of intensity of a given feature, and 5 meant a very intense intensity of a given feature. During the evaluation, the staff checked the time and instructed panellists to evaluate the sensory attribute in accordance with the list of sensory attributes.

2.2. Determination of the color in rooibos and honeybush samples

The color parameters of rooibos and honeybush beverages were determined with a Konica Minolta CR-400 colorimeter. The determination was performed in the CIE system, which is based on measuring the three trichromatic components (L*,a*,b*). All the parameters were measured three times in every sample.

2.3. Statistical analysis

Statistical analysis of the results included the calculation of basic measures such as the mean value. Statistical significance of differences between rooibos and honeybush samples were determined using STATISTICATM12. The Kruskal-Wallis were performed for testing significant group differences followed by a post hoc U Manna-Whitney test, to determine which groups differed significantly. Principal compound analysis (PCA) was used to discriminate between varieties. PCA using the correlation matrix was conducted using STATISTICATM12 to visualize and elucidate the relationships between the samples. Agglomerative hierarchical clustering (AHC) was also performed to identify the relationships between test samples. A dendrogram of AHC was used to visually present information regarding clustering based on dissimilarity between rooibos and honeybush samples. Differences were considered statistically significant when $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The principle of colorimetry is based on the tristimulus method, i.e., each color is the combination in different proportions of red, green and blue. For one color, the colorimeter gives the percentages of the three primary colors. Parameter L* indicates lightness, its value extended from 0 (black) to 100 (white). Parameters a* and b* are the chromaticity coordinates. The a* and b* indicate color directions: + a* is the red direction, − a* is the green direction, + b* is the yellow direction, and − b* is the blue direction. The center is achromatic, as the a* and b* values increase and the point moves out from the center, the saturation of the color increases [Korifi et al. 2013]. The mean and statistic data of color parameters concentrations determined in the samples of rooibos and honeybush are presented in Table 1.

Table 1. Mean values of the color parameters (CIEL*a*b*) for selected rooibos and honeybush beverages

Tabela 1. Wartości średnie parametrów barwy (CIEL*a*b*) dla wybranych naparów rooibos i honeybush

Parameter	Rooibos			Honeybush		
	R_K	**R_LN**	R_S	H_BM	H_CnH	H_SL
L*	25.52	25.79	24.56	30.28	32.81	26.62
K-W test	KW-H(5;12) = 10.53; p = 0.06					
a*	12.83	10.68	10.98	10.62	6.02	9.05
K-W test	KW-H(5;12) = 10.08; p = 0.07					
b*	9.88	10.26	8.02	16.27	16.73	11.55
K-W test	KW-H(5;12) = 10.23; p = 0.07					

Source: own study.

As can be seen in Table 1, the mean parameter L* was higher for the honeybush samples, it ranged from 26.62 (Simon Lévelt) to 32.81 (H_CnH), demonstrating a high whitening value, as expected since L* represents lightness. Rooibos samples were darker than honeybush samples. The L* parameter for these beverages ranged from 24.56 (R_S) to 27.79 (R_LN). It can also be noted that the rooibos samples showed higher values of the parameters a* (from 10.68 to 12.83 Lord Nelson and Kawon samples respectively) presenting a color closer to red. Whereas the b* parameters were lower for all samples of rooibos (from 8.02 to 10.26 for Samowar and Lord nelson samples respectively) demonstrating a loss of yellow color. The Kruskal-Wallis analysis were applied in order to determine whether the samples were different concerning to kind of beverages. Significant differences (confidence level of 95%) were not observed for most color parameters, do not indicating the possibility to identify the rooibos and honeybush samples by statistical analysis.

Despite not identifying statistically significant differences, the relationships between the tested infusions have been observed. Some rooibos and honeybush samples were grouped based on their color parameters. For instance, all rooibos samples are grouped closely together on the plot. The honeybush H_SL infusion was also classified in the same group. These results were consistent with the findings of the Agglomerative Hierarchical Clustering (AHC). The dendrogram (Fig. 1.) provides the visual data regarding the clustering of the 6 type of rooibos and honeybush samples, as well as the relationships between samples.

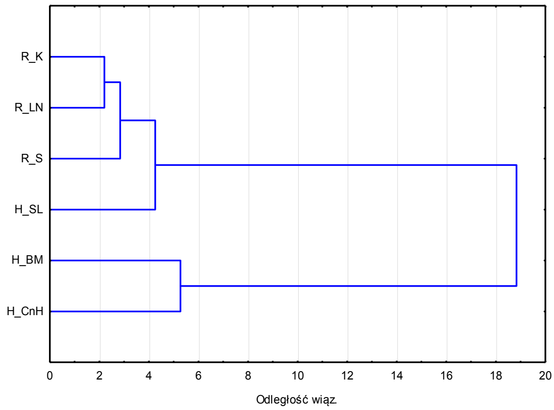

Fig. 1. The dendrogram drawn by agglomerative hierarchical clustering (AHC) of a total of 6 type of rooibos and honeybush (CIELab)

Rys. 1. Dendrogram (AHC) grupujący 6 próbek rooibos i honeybush

Source: own study.

Based on the dissimilarity of color parameters between rooibos and honeybush samples, the 3 rooibos samples were divided into one cluster. It is interesting to note that honeybush (H_SL) samples were included in the same cluster as rooibos (probably due to the similar value of the L* parameter), whereas the two honeybush samples (H_BM and H_CnH) were separated into one different cluster. These results imply that the color parameters are different to rooibos and honeybush.

The next stage was color evaluation of rooibos and honeybush beverages by the consumers. The results of this research are presented in Table 2.

Statistical analysis of the obtained results for all rooibos and honeybush infusions showed a statistically significant difference in intensity for the majority of the tested color attributes (brick, red, brown, orange). Only for one color descriptor (amber) there was no statistically significant difference between rooibos and honeybush infusions (Tab. 1).

The infusions prepared with *Aspalathus Linearis*, in the consumers' opinion, were characterized by a more intense saturation of brick, red and brown. While honeybush infusions were characterized by greater brightness and higher saturation of orange.

Analysis of data obtained for individual rooibos infusions showed statistically significant relationships only for the intensity of red color and the brightness of the beverages.

Table 2. Mean values of sensory evaluation of color attributes for some selected rooibos and honeybush beverages

Tabela 2. Wartości średnie sensorycznej oceny barwy wybranych naparów rooibos i honeybush

Product		Intensity of assessed color attributes					
		brick	red	brown	amber	orange	brightness
Rooibos	R_K	3.60[a]	3.40[a]	2.20[a]	2.47[a]	1.60[a]	0.33[a]
	R_LN	2.80[a]	2.30[b]	2.47[a]	3.33[a]	2.43[a]	1.10[b]
	R_S	3.20[a]	2.97[a]	2.17[a]	2.90[a]	1.67[a]	0.73[b]
	K-W test rooibos (H(2,90))	H=4.79 p=0.09	**H=7.16 p=0.03**	H=0.77 p=0.67	H=4.10 p=0.12	H=4.93 p=0.08	**H=11.3 p=0.03**
Honeybush	H_BM	1.40[a]	0.80[a]	2.07[a]	2.83[a]	2.47[a]	2.60[a]
	H_CnH	0.37[b]	0.40[b]	0.97[b]	2.53[a]	2.23[a]	4.30[b]
	H_SL	1.90[c]	1.23[a]	2.07[a]	3.53[b]	2.60[a]	2.07[a]
	K-W test honeybush (H(2,90))	**H=25.0 p=0.00**	**H=9.45 p=0.01**	**H=16.5 p=0.00**	**H=7.22 p=0.03**	H=0.72 p=0.69	**H=30.1 p=0.00**
K-W test rooibos – honeybush (H(5,180))		**H=79.4 p=0.00**	**H=71.5 p=0.00**	**H=22.0 p=0.00**	H=11.1 p=0,051	**H=12.4 p=0,03**	**H=98.5 p=0.00**

Data in bold font are statistically significantly different (p ≤ 0,05); a-c – different letters in the same column indicate statistical differences between mean values at p ≤ 0,05.

Source: own study.

In the case of infusions prepared from Cyclopia spp., data analysis showed statistically significant relationships for all color descriptors with the exception of orange color. Honeybush beverages (H_BM and H_SL) were characterized most of all by a more intensive saturation of all tested colors.

Also for color parameters obtained from sensory evaluation there can be seen the relationship between the tested infusions. Moreover, based on the results obtained and the dependencies existing between them, we can group the different brands of rooibos and honeybush (Fig. 2).

The dendrogram, similar to the previous one, provides the visual data regarding the clustering of the 6 type of rooibos and honeybush samples, as well as the relationships between samples. Based on the dissimilarity of sensory evaluation parameters (especially color parameters) between rooibos and honeybush samples, the 3 rooibos samples were divided into one cluster and 3 honeybyush samples also were divided into one, quite different, cluster.

These results imply that the color parameters described by consumers can be good tool to differentiate the rooibos and honeybush beverages.

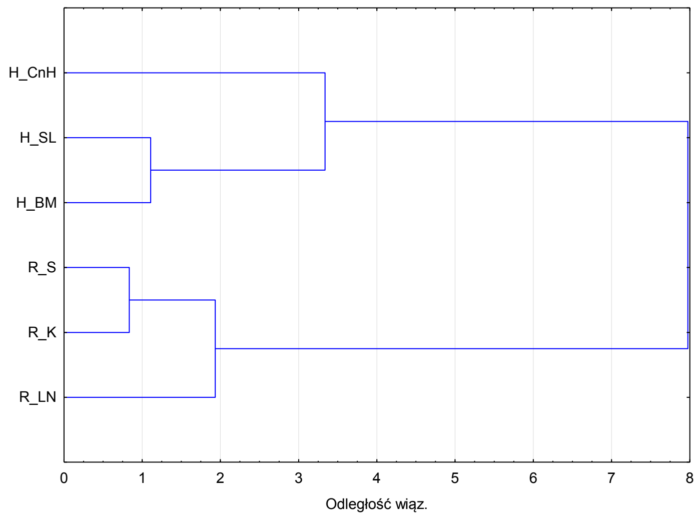

Fig. 2. The dendrogram drawn by agglomerative hierarchical clustering (AHC) of a total of 6 type of rooibos and honeybush according to sensory quality evaluation
Rys. 2. Dendrogram (AHC) grupujący badane rooibos i honeybush według parametrów jakości sensorycznej

Source: own study.

To display the relationships between attributes, as well as between individual samples the standardized PCA plots using the correlation matrix are commonly used in sensory analysis. Most of the sensory evaluation and CIEL*a*b* attributes were closely associated with one another (Fig. 3a), indicating that these notes were perceived similarly by the consumers color evaluation as well as by the instrumental measurement of color.

The PCA loadings plot (Fig. 3a) displays the positioning of, and association between the rooibos and honeybush attributes. This plot shows the separation between two groups of attributes, as amber, brown, red, brick color, that positively correlate with a* parameter (except for amber color) and second sensory characteristics – brightness of color, that is closely associated with L* and b* color parameters.

The positioning of 3 rooibos and 3 honeybush samples relative to each other is reflected by the corresponding scores plot (Fig. 3b). It is evident that distinct clusters are formed based on the quality grade of the samples. Nevertheless, all rooibos samples are separated from honeybush samples along the first Principal Component or Factor 1 (F1).

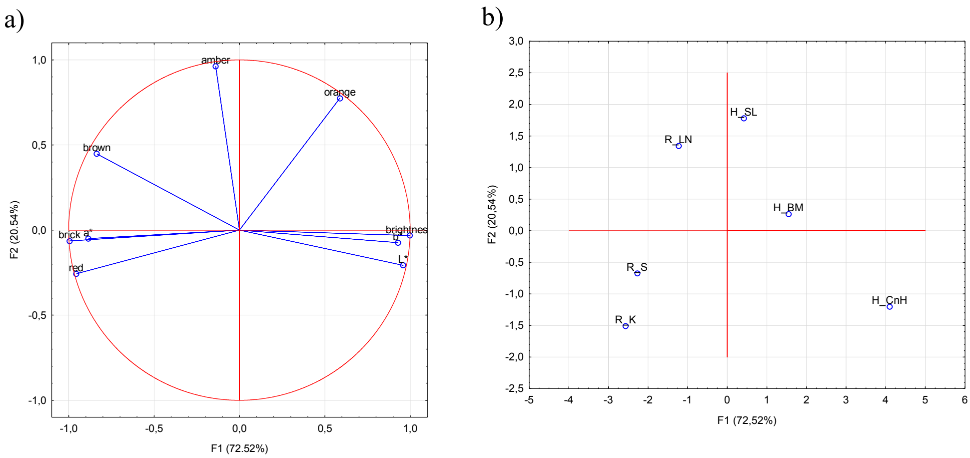

Fig. 3. PCA loadings (a) and scores (b) plots showing the positioning of 9 sensory and color parameters attributes and 3 rooibos and 3 honeybush samples, respectively

Rys. 3. Wykres PCA ładunków (a) i punktów (b) pozycjonujący 9 atrybutów sensorycznych i parametrów barwy dla trzech próbek rooibos i honeybush

Source: own study.

This indicates that rooibos samples associate with the color attributes, such as amber, red, brown in the left quadrants, while honeybush samples correspond to the orange color and brightness of color and also from L* and b* parameters in the right quadrants. This indicates that the sensory quality of a sample (especially color) of rooibos beverages is not necessarily in accordance with the sensory quality of a sample of honeybush beverages, and that there is considerable variation in sensory characteristics within each kind of products. For instance, some of the rooibos samples (e.g., R_K and R_LN) were found to have stronger brick, red and brown notes, whereas honeybush samples (e.g., H_BM and H_CnH) illustrated a prominent and thus noticeable orange color (Tab. 2).

Furthermore, from 9 sensory attributes used for descriptive analysis only 4 differed significantly between the four grades. The differences in average attribute intensities between the quality grades are displayed in Tab. 1 and Tab. 2. On average, rooibos samples are associated with higher intensities for a* and L* parameters, and with lower intensities for yellow color and less beverages brightness. Honeybush samples on the other hand may be described according to L*a*b* parameters as more yellow (orange).

4. CONCLUSIONS

During the last few years consumers have renewed interest in authenticity of foods. The use of brand indications (for instance, roooibos and honeybush) allows producers to obtain market recognition and often a special (premium) price. False use of product indications by unauthorised parties is detrimental to consumers and legitimate producers. From this point of view, the development of new and increasingly sophisticated techniques for determining the kind of products is highly desirable for consumers, retailers and administrative authorities. It is an analytically challenging problem that is currently the focus of much attention within commodity sciences. This is the one of the first study providing an overall profile of the color parameters of rooibos and honeybush to identify these products.

The results point out that these models should be applied to distinguish rooibos from honeybush, but more analysis is necessary. However, the present models can be considered as not very stable but valid for the prediction of color parameters in distinguish these two kinds of product, mainly for sensory evaluation of color beverage parameters and also for L*, a* and b* parameters.

The classification of rooibos and honeybush according to the beverages parameters was possible by measuring sensory color parameters and less by measuring color parameters in CIEL*a*b* system. It is noteworthy, that the samples of rooibos were characterized by different value of color parameters than honeybush ones.

The results presented in this paper indicate that samples of rooibos were very similar and were characterized by more intensive brick, red and brown color than honeybush beverages. The results of such studies would be important with regard to the current distinguishing the rooibos from honeybush. The newly developed relations would be valuable tools for such research purposes. The problem is really topical and needs further studies in the future and it is worth verifying obtained results on a larger number of samples in the future.

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