Verification of rapid method for estimation of added food colorant type in boiled sausages based on measurement of cross section color

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Abstract. During the previous development of a chemometric method for estimating the amount of added colorant in meat products, it was noticed that the natural colorant most commonly added to boiled sausages, E 120, has different CIE-LAB behavior compared to artificial colors that are used for the same purpose. This has opened the possibility of transforming the developed method into a method for identifying the addition of natural or synthetic colorants in boiled sausages based on the measurement of the color of the cross-section. After recalibration of the CIE-LAB method using linear discriminant analysis, verification was performed on 76 boiled sausages, of either frankfurters or Parisian sausage types. The accuracy and reliability of the classification was confirmed by comparison with the standard HPLC method. Results showed that the LDA + CIE-LAB method can be applied with high accuracy, 93.42 %, to estimate food color type in boiled sausages. Natural orange colors can give false positive results. Pigments from spice mixtures had no significant effect on CIE-LAB results.

1. Introduction

Technological processes and components used in manufacturing have a major influence on sensory properties of meat products, and therefore, on acceptance by consumers [1]. Meat products have a characteristic color, which could make them acceptable or not at first glance. The color of meat products can be adjusted by addition of natural and/or synthetic dyes. This addition not only provides a uniform color of the products from batch to batch, but also allows the replacement of high-quality components with ones of lower quality, fraud and deception of the consumer. Usage of synthetic and some natural food dyes can have adverse reactions on human health, especially in children and sensitive adults [2-5]. Hence, their use in meat products is regulated [6-8].

Color measurement of meat and meat products using the CIE (Commission Internationale de l'Eclairage) L*a*b* system (CIE-LAB) is in common use, along with sensory evaluation [9-10]. Instrumental color measurement is independent of auditors' subjective impressions. The widespread application of CIE-LAB measurement can be explained due to the uniform distribution of color and similarity to color perception of the human eye. The CIE-LAB method along with chemometrics has been applied to predict color properties of food during accelerated shelf-life studies, recipe changes, package and storage conditions and so forth [11-14]. CIE-LAB measurements cannot be applied for direct determination of added food colorants in meat products without chemometrics, because other coloring materials, like paprika and chili from spice mixtures, and the color of the constituent meat, contribute to the overall color [15]. Reliability of measurements depends on color homogeneity of the analyzed surface, and in the case of meat products, freshly-sectioned surfaces are required. Measurement of cross-sectional color of fine-grained products like frankfurters and Parisian sausage gives accurate and repeatable results.

It is not necessary to emphasize the need for rapid methods and techniques for food color monitoring throughout the entire food supply chain, from the manufacturers to retailers. In order to contribute to this topic, the goal of this study was to investigate the possibility of re-purposing a previously developed method [15] to distinguish the addition of synthetic or natural food colorants in boiled sausages with even cross-sectional color. After initial adjustment, the method was verified on retail products, and results of the CIE-LAB method were compared with results of HPLC method [15].

2. Materials and methods

Reagents, meat products for development and calibration of the CIE-LAB method, sensory and HPLC analysis, and CIE-LAB measurements were performed according to the procedures previously described [15]. Meat products for verification (76 boiled sausages, either frankfurters or Parisian sausages) were obtained from importers, exporters or retailers.

2.1. Statistical analysis

Techniques and methods previously described were used for data analysis [15]. Principal component analysis (PCA) and linear discriminant analysis (LDA) [16] were used to determine the existence of data grouping based on CIE-LAB measurement as well as to produce the model for determination of the colorant type in boiled sausages. MS Office Excel and JMP 10 Statistical Discovery (SAS Institute, SAD) were used for data processing and all calculations.

3. Results and discussion

Ten fine-grained boiled sausage products with different food colorants at different concentration levels produced in an industrial facility [15] were used as a training set. Sixty results for CIE-LAB measurements were used for analysis. First of all, PCA was applied to determine grouping either by type or colorant concentration level. Graphically represented results of PCA are shown in Figure 1.

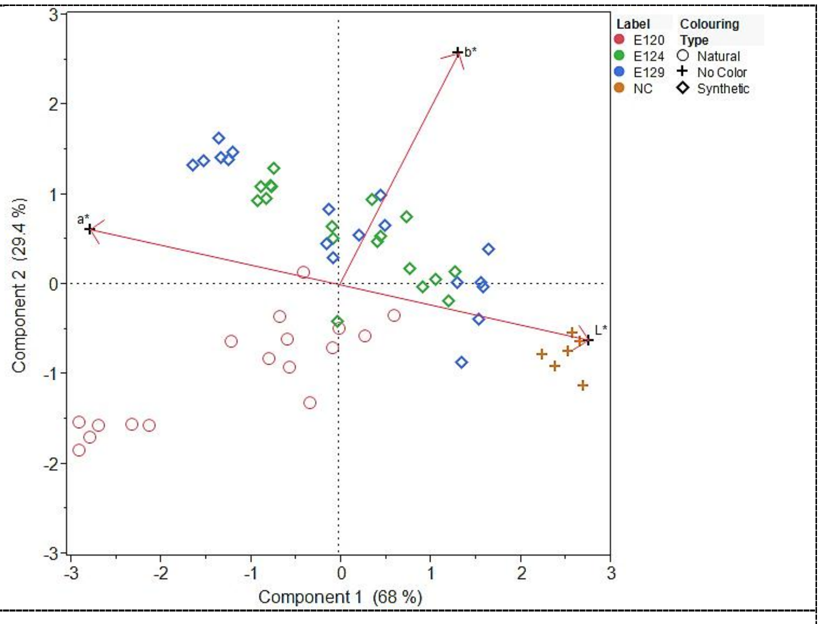

Figure 1. PCA results for the training set of ten boiled sausage products

Principal components 1 (PC1) and 2 (PC2) explained 89.4 % of total variance. Results showed that the main factors for grouping results by dye concentration level were the amount of red (a*) and lightness (L*). As the dye concentration increased, a* also increased, while L* values decreased. For sausages without added colorant (NC, No Colorant, orange cross in Figure 1) the luminance value (lightness, L*) was the most relevant grouping factor. Synthetic colorants (E 124 and E 129) were separated from natural dye (E 120) by b*, the yellowness value. Values of b* increased with increasing concentrations of synthetic dye, while increases in the amount of added natural colorant decreased b* values. Values of L* and a* showed more significant differences with concentration changes of natural than of synthetic dyes. In conclusion, PCA results for the training set indicated that sausages could be separated into three groups according to type of added food colorant – with no added colorant, with synthetic colorant and with natural colorant.

Re-purposing the CIE-LAB method to identify the type of colorant added to boiled sausages with homogenous section color involved LDA of the training set data. LDA was selected because it is suitable for calibration with categorical output variables. Results of LDA are shown in Table 1.

Table 1. LDA results for the training set of ten boiled sausage products

Counts: Actual Rows by Predicted Columns	Natural	No Color	Synthetic
Natural	17	0	1
No Color	0	6	0
Synthetic	1	2	33

Actual groups in the training set are given by rows, and predicted by columns in Table 1. Only 5 of the 60 measurements resulted in a misclassification. In the natural group, 1 was misclassified; in the group with no colorant, 0 were misclassified, and; 3 were misclassified in the group with synthetic colorants. The modified CIE-LAB method produced 91.67 % accurate prediction in the training set of boiled sausages. By groups, the percent of accurate prediction was as follows: 94.44 % in the natural colorants group, 100 % in the group without added colorant, and 91.67 % in the synthetic colorants group.

The verification set consisted of 76 samples of boiled sausages. Sensory audit of cross-section color was performed simultaneously, along with CIE-LAB measurements. Food dye content and type were determined and confirmed by the HPLC-DAD method [15]. Results of colorimetric, sensory and chromatographic determinations were compared and applied for estimation of CIE-LAB method validity (Table 2).

Table 2. Verification results for 76 boiled sausages from retail, export or import using CIE colorimetry + LDA to predict the type of colorant added to the foods

No	Type[a]	CIE colorimetry			Sensory	Probability (%)			Estimated dye (CIE)	Dye type (HPLC)	Correctness of classification
		L*	a*	b*		NC[b]	Nat[c]	Syn[d]			
1	FR	67.60	15.63	16.12	3.08	0.00	0.00	100.00	Syn	E 120	-
2	PS	74.71	14.30	13.78	4.92	100.00	0.00	0.00	NC	ND	+
3	PS	75.79	13.08	11.73	4.67	100.00	0.00	0.00	NC	ND	+
4	PS	66.25	16.21	20.73	2.83	0.00	0.00	100.00	Syn	E 129	+
5	PS	68.11	15.14	17.63	3.00	0.00	0.00	100.00	Syn	E 129	+
6	PS	72.78	8.83	13.67	3.25	80.18	11.87	7.95	NC	ND	+
7	PS	63.97	19.29	14.92	3.25	0.00	64.63	35.37	Nat	E 120	+
8	PS	69.01	15.61	12.88	4.83	0.00	99.91	0.09	Nat	E 120	+
9	PS	72.24	14.77	11.96	4.92	87.14	12.85	0.01	NC	ND	+
10	FR	68.63	16.57	11.98	3.58	0.00	100.00	0.00	Nat	E 120	+
11	FR	66.96	15.71	13.65	3.00	0.00	99.76	0.24	Nat	E 120	+
12	FR	68.41	14.31	13.90	2.50	0.00	94.10	5.90	Nat	E 120	+
13	FR	69.48	15.26	14.97	3.25	0.00	0.08	99.92	Syn	E 129	+

No	Type[a]	CIE colorimetry			Sensory	Probability (%)			Estimated dye (CIE)	Dye type (HPLC)	Correctness of classification
		L*	a*	b*		NC[b]	Nat[c]	Syn[d]			
14	FR	68.02	15.14	15.57	2.25	0.00	0.03	99.97	Syn	E 110	+
15	PS	66.83	17.01	12.56	4.63	0.00	100.00	0.00	Nat	E 120	+
16	PS	71.35	14.87	13.04	4.75	22.23	68.70	9.07	Nat	E 120	+
17	PS	68.92	14.78	17.12	3.38	0.00	0.00	100.00	Syn	E 129	+
18	FR	62.53	19.83	14.50	3.38	0.00	99.67	0.33	Nat	E 120	+
19	PS	63.60	19.16	15.68	3.00	0.00	3.02	96.98	Syn	E 110	+
20	FR	67.84	15.86	14.32	4.00	0.00	45.62	54.38	Syn	E 100	-
21	FR	68.82	15.45	14.74	4.13	0.00	1.13	98.87	Syn	E 100	-
22	PS	63.91	16.20	13.63	3.00	0.00	100.00	0.00	Nat	E 120	+
23	PS	65.44	18.26	16.45	3.50	0.00	0.00	100.00	Syn	E 110	+
24	FR	61.03	18.94	12.44	4.00	0.00	100.00	0.00	Nat	E 120	+
25	FR	66.83	17.41	12.76	4.00	0.00	100.00	0.00	Nat	E 120	+
26	FR	63.31	18.91	12.49	4.50	0.00	100.00	0.00	Nat	E 120	+
27	FR	63.95	18.43	10.52	3.50	0.00	100.00	0.00	Nat	E 120	+
28	PS	60.61	18.27	12.34	3.50	0.00	100.00	0.00	Nat	E 120	+
29	FR	59.57	20.55	13.48	3.50	0.00	100.00	0.00	Nat	E 120	+
30	PS	61.47	19.67	12.51	3.50	0.00	100.00	0.00	Nat	E 120	+
31	PS	66.60	18.00	11.42	2.50	0.00	100.00	0.00	Nat	E 120	+
32	PS	63.65	17.24	13.96	3.50	0.00	99.99	0.01	Nat	E 120	+
33	PS	66.22	17.15	14.44	3.00	0.00	75.17	24.83	Nat	E 120	+
34	FR	62.76	15.79	16.00	2.50	0.00	28.47	71.53	Syn	E 110	+
35	PS	63.94	18.70	17.01	3.00	0.00	0.00	100.00	Syn	E 100	-
36	PS	67.17	17.86	12.88	3.50	0.00	99.98	0.02	Nat	E 120	+
37	PS	68.95	13.43	12.32	3.50	0.00	100.00	0.00	Nat	E 120	+
38	PS	72.31	13.90	14.32	-	93.96	0.00	6.03	NC	ND[e]	+
39	PS	63.48	16.31	13.20	-	0.00	100.00	0.00	Nat	E 120	+
40	PS	66.63	15.94	13.01	4.00	0.00	100.00	0.00	Nat	E 120	+
41	PS	60.58	19.56	12.40	4.00	0.00	100.00	0.00	Nat	E 120	+
42	FR	65.47	12.23	13.89	4.00	0.00	100.00	0.00	Nat	E 120	+
43	FR	65.91	13.01	13.81	4.50	0.00	99.99	0.01	Nat	E 120	+
44	FR	64.35	14.10	13.85	3.50	0.00	100.00	0.00	Nat	E 120	+
45	PS	65.70	15.36	13.38	4.00	0.00	100.00	0.00	Nat	E 120	+
46	PS	66.23	17.24	13.76	3.50	0.00	99.56	0.44	Nat	E 120	+
47	PS	63.68	17.11	13.24	3.00	0.00	100.00	0.00	Nat	E 120	+
48	PS	65.34	16.67	14.03	3.50	0.00	99.73	0.27	Nat	E 120	+
49	PS	69.24	14.00	13.79	4.00	0.00	89.36	10.64	Nat	E 120	+
50	PS	65.91	13.01	13.81	3.50	0.00	99.99	0.01	Nat	E 120	+
51	PS	63.69	17.08	14.14	3.50	0.00	99.97	0.03	Nat	E 120	+
52	PS	61.03	17.93	14.19	4.00	0.00	100.00	0.00	Nat	E 120	+
53	FR	66.92	16.54	13.58	4.50	0.00	99.70	0.30	Nat	E 120	+
54	FR	60.83	19.89	13.11	4.00	0.00	100.00	0.00	Nat	E 120	+
55	PS	60.58	19.71	12.49	4.00	0.00	100.00	0.00	Nat	E 120	+
56	PS	63.69	17.08	14.14	3.50	0.00	99.97	0.03	Nat	E 120	+
57	FR	65.47	12.23	13.89	3.50	0.00	100.00	0.00	Nat	E 120	+
58	PS	61.03	17.93	14.19	3.00	0.00	100.00	0.00	Nat	E 120	+
59	FR	65.91	13.01	13.81	4.50	0.00	99.99	0.01	Nat	E 120	+
60	FR	64.35	14.10	13.85	4.00	0.00	100.00	0.00	Nat	E 120	+
61	PS	65.70	15.36	13.38	4.00	0.00	100.00	0.00	Nat	E 120	+
62	PS	66.23	17.24	13.76	3.50	0.00	99.56	0.44	Nat	E 120	+
63	PS	63.68	17.00	13.24	3.50	0.00	100.00	0.00	Nat	E 120	+
64	PS	65.34	16.67	14.03	4.50	0.00	99.73	0.27	Nat	E 120	+
65	PS	69.24	13.97	13.79	4.00	0.00	89.64	10.36	Nat	E 120	+
66	PS	65.39	16.33	12.96	4.00	0.00	100.00	0.00	Nat	E 120	+
67	PS	67.62	15.86	15.44	4.00	0.00	0.09	99.91	Syn	E 110	+
68	FR	60.63	19.98	12.76	4.00	0.00	100.00	0.00	Nat	E 120	+
69	PS	64.28	15.34	15.26	3.00	0.00	78.18	21.82	Nat	E 120	+
70	PS	61.04	16.80	15.41	3.50	0.00	99.56	0.44	Nat	E 120	+
71	FR	66.91	16.35	13.67	4.50	0.00	99.56	0.44	Nat	E 120	+
72	PS	61.47	16.02	15.44	3.50	0.00	99.34	0.66	Nat	E 120	+

No	Type[a]	CIE colorimetry			Sensory	Probability (%)			Estimated dye (CIE)	Dye type (HPLC)	Correctness of classification
		L*	a*	b*		NC[b]	Nat[c]	Syn[d]			
73	PS	68.32	15.09	13.18	3.50	0.00	99.90	0.10	Nat	E 120	+
74	PS	65.89	15.23	14.68	3.50	0.00	87.41	12.59	Nat	E 120	+
75	PS	68.28	13.43	12.48	3.50	0.00	100.00	0.00	Nat	E 120	+
76	PS	66.54	14.90	15.45	3.00	0.00	1.72	98.28	Syn	E 100	-

[a] FR = frankfurter; PS = Parisian sausage
[b] NC = no colorant
[c] Nat = natural colorant
[d] Syn = synthetic colorant
[e] ND = none detected

Verification of the method for estimating the type of food dye added to boiled sausages based on CIE-LAB colorimetry measurements was conducted by comparison of correctly classified vs. misclassified sausages. The overall estimated accuracy of verification was 93.42 %. Five of 76 sausages were misclassified, of which four were confirmed by HPLC-DAD as containing the orange natural food dye, curcumin (E 100). Considering that the b* value was the main discriminant factor for differentiation of synthetic and natural food colorants used in the training set, and that in orange food colorants, the amount of yellow (b*) is significantly higher than in red food colorants, it is easy to deduce why the use of E100 gave false positive results for synthetic dyes. In the case of the other orange colorant detected in the sausages (Sunset yellow FCF, E 110), it was correctly classified since it is a synthetic colorant.

Similarly to the training set, no sausages without added food colorant were misclassified in this verification of 76 sausages. The absence of misclassified samples in the group of sausages with no added color points to the fact that pigments from spice mixtures had no significant effect on CIE-LAB determination.

4. Conclusion

CIE-LAB measurement is a fast, nondestructive method for *in-situ* determination of product color characteristics and it can be applied, with chemometrics, to determine the amount of colorants in meat products. The fine-grained boiled sausages studied, with even cross-sectional color, gave repeatable CIE-LAB measurement results [15].

The previously developed method for estimation of added food dye amount based on multiple linear regression (MLR) and CIE-LAB measurements was successfully transformed into a method for estimation of added colorant type (natural, synthetic, or no colorant) by substitution of color concentration with color type and use of LDA in place of MLR.

Verification results showed that the LDA + CIE-LAB method could be applied with high accuracy to estimate food colorant type in boiled sausages. However, natural orange colorants can be falsely categorized as synthetic colorants. Sausages with added natural red colorant, except for one sample, were all correctly classified, as were sausages without added color.

Pigments from spice mixtures had no significant effect on CIE-LAB results.

This CIE-LAB method can be used as a complementary method alongside HPLC determinations and sensory analysis for rapid estimation of the type of added colorant in boiled sausages as an important control factor in food supply chain.

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