6.05

Utilization of animal blood for human consumption. Sensory evaluation and measurement of surface colour of meat products, and determination of hemoglobin and myoglobin in raw materials.

ERIK SLINDE, JAN MIELNIK, MAGNI MARTENS AND ARNE TENNINGEN

Norwegian Food Research Institute, P.O.Box 50, N-1432 Aas-NLH, Norway.

In human metabolism is iron of fundamental importance and one of its main function is the transport of oxygen. Yet millions of people suffer from iron-deficiency anemia, and especially women of child-bearing age. Blood is particularly rich in heme iron, which is the biologically most available form of iron. In addition blood is also a good protein source. Attempts to add back blood to meat products should therefore be taken.

Traditional blood products have blood as the main component. Such products have a black colour due to the high amount of hemoglobin (Hb), and a characteristic taste due to the high amount of blood. The amount of pigment in a recipe reflects its colour, and the main contributor to colour is the chromophoric group heme, found in the muscle pigment myoglobin (Mb) and in the blood pigment Hb. The amount of Mb varies in different animals and among different muscles, and amount to approximately 3.5 mg/g in pigs, 5 mg/g in beef, 6 mg/g in horse and seal, and 8 mg/g in blue whale (George et al., 1971). Beef blood contains approximately 150 mg/g Hb and the amount in beef varies from 0,2-2,0 mg/g (Warriss and Rhodes, 1977). Thus, all meat products contain a small amount of blood. The molecular weight of Hb is approximately four times that of Mb (65,000 vs. 17,000). Since Mb contain one and Hb four heme groups per molecule is the colour intensity proportional to the weight amount of either Hb or Mb added to a recipe. Addition of 1% bovine blood to a recipe increases the pigment content approximately 1.5 mg/g. Previous experiments (Slinde and Martens, 1982) have shown that darkening is one of the main problems when blood is used in meat products. A method that makes possible an objective assessment of surface colour is necessary for the evaluation of new recipes that utilize blood. Since colour is determined by the amount of pigment present in a recipe a quantification of Hb and Mb in the raw materials is necessary.

The aim of the present study was: a) Add blood to an emulsion product and evaluate the changes in sensory properties. b) Measure colour properties of the processed meat product and calculate the visual colour parameters. c) Determine the amount of Hb and Mb present in the raw materials.

Materials and Methods

Meat loafs were produced according to the following basic recipe (100 kg): 20 kg cow meat (18.4% protein, 15% fat, 66% water), 19 kg beef meat (17.3% protein, 21% fat, 61.5% water), 12 kg fat (6.8% protein, 68.5% fat, 24% water), 0.5 kg soy protein, 1.7 kg salt, 4 kg potato starch, 0.3 kg sesonings and 42.5 kg skim milk. To this amount were added blood and/or nitrite and ascorbic acid as indicated in the figures. Approximately one kg meat loafs were heated in aluminium forms to a core temperature of 78-79°C.

Sensory evaluation was performed by a well trained laboratory panel of 12 persons. 3 replicates from each of the six meat loafs were judged with regard to the following sensory properties, using ninepoint scales as indicated in the parantheses: a) Colour intensity of a freshly cut surface (1 = very light, 9 = very dark). b) Total impression of colour (1 = very bad, 9 = very good). c) Meat flavour (1 = none, 9 = very strong). d) Off-flavour (1 = none, 9 = very strong). e) Total impression of flavour (1 = very bad, 9 = very good). f) Hardness (1 = not hard, 9 = very hard). g) Juiciness (1 = very dry, 9 = very juicy. h) Coarsness (1 = not coarse, 9 = very coarse). i) Total impression of texture (1 = very bad, 9 = very good). The temperature of the samples taken from each of the meat loafs when evaluated was 50 ± 1°C. The samples were randomized within each replicate and also with respect to the order of serving to the different judges. Six samples were served in each session together with a reference sample without blood added. The panel was instructed to judge one sample at a time in comparison with the reference sample. The scores for the reference sample on the ninepoint scales were agreed upon in initial evaluation sessions, and these reference scores were printed on the evaluation sheets. The judges were not told that blood had been added to the product. Concerning statistical analysis of the sensory data, standard error of the mean of 3 replicates for each sensory variable was calculated. To test the statistical significant differences between the samples a studentized range test was performed.

Colour measurements. The reflectance of the cut surface of the meat loafs was measured using the integrating sphere attachment of the Shimadzu UV-300 spectrophotometer (Shimadzu Sesakusho Ltd., Kyoto, Japan). A glass plate was used to cover the sample and the MgO that was used as white reference.

The spectrophotometer is connected to a computer (NORD 10/S, Norsk Data A/S, Oslo, Norway), with a CAMAC process interface for direct reflectance measurements. A DC-voltage corresponding to the reflectance is amplified and transmitted to the computer through a multiplexer and a 12 bit analogue-to-digital converter. The interface includes digital input signals to indicate start and stop of the spectrophotometer. The current wave length is not transmitted, but is computed based on parameters given by the operator. The computer has a harddisk for data storage and a graphic terminal for spectrum plotting. The software includes programs for the following operations: - operator communication/parameter definition - data aquisition/calibration - storing - plotting - statistics - colour computations. The operator defines for each spectrum a set of parameters describing the material to be sampled and how to do it. The parameters include wave length limits, scanning speed and sample interval (the spectrum is sampled at predefined intervals). Time and date of recording are also saved as parameters for each scan. Based on these parameters the sampling periode is computed. For each selected wave length the reflectance value is corrected using calibration values for 0% and 100% reflectance previously recorded. This correction is performed to reduce wave length depending errors in the spectrophotometer. To handle the storing of a large amount of data from the spectrophotometer a filesystem has been developed. Each spectrum is stored together with a set of parameters describing the measurement conditions. Each user has his own version of the filesystem and within each filesystem the spectra are

referred to by a reference number which is later used to retreive the spectrum for computation or plotting. The calibration spectra for 0% and 100% reflectance are also stored. Different spectra may be plotted and compared on the graphic terminal as a function of wave length. The operator specify the reflectance scale and the wave length area of interest. Graphical plots may be copied using a hardcopy unit giving paper copies. Computing mean spectra from several spectra has been implemented to reduce the effects of random noise. The spectra must cover the same wave lengths, and the mean spectrum is treated as an ordinary spectrum with respect to storage, plotting and colour computations. Colour computations are all based on the CIE-tristimulus values for a selected light source. The system includes data for A, B, C, D65 and equal-energy light-sources. Other lightsources are easily implemented. For each colour computation the operator specifies for which lightsource the computation should be done. Several colour systems may be computed based on the same spectrum, and systems today included are (Hunter, 1975): - CIE tristimulus values X, Y and Z - CIE trichromaticity coefficients x, y and z - CIE λ_d, P_e and P_c - CIE U*, V* and W* - CIE-1976 L*, a* and b* - CIE-1976 L*, u* and v* - McAdams Y, u and v - Hunter L', a' and b'.

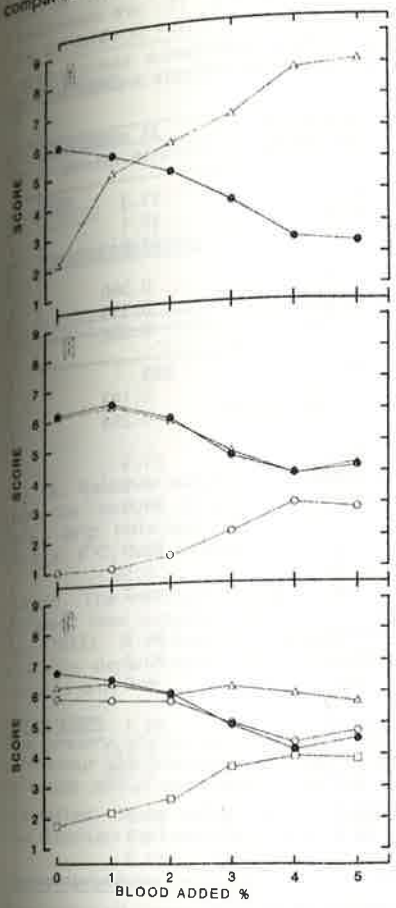

Fig. 1. Sensory evaluation (mean of score of 12 judges x 3 replicates) of meat loafs when small amounts of blood are added. s=average standard error of the mean for 6 samples. A: (Δ) colour intensity (s=0.14), (●) total impression of colour (s=0.17). B: (Δ) meat flavour-by-mouth (s=0.13), (o) off-flavour (s=0.13), (●) total impression of flavour (s=0.12). C: (o) hardness (s=0.12), (Δ) juiciness (s=0.10), (□) coarsness (s=0.12), (●) total impression of texture (s=0.11).

High performance liquid chromatography using a Bio-Sil TSK-125 (300 x 7,5 mm) molecular sieve column was used to determine the amount of Hb and Mb in the raw materials. The samples were extracted, cleared by sedimentation in a Sorval preparative ultracentrifuge (20 000 rpm, 30 min), and injected directly through a Bio-Sil TSK guard column (75 x 7.5 mm) in order to protect the separation column. The extraction buffer, as well as the mobile phase consisted of 65 mM K-phosphate buffer, pH 6.8 containing 0.1 M KCl, and a flow rate of approximately 1 ml·min^{-1} was used (Kryvi et al., 1981).

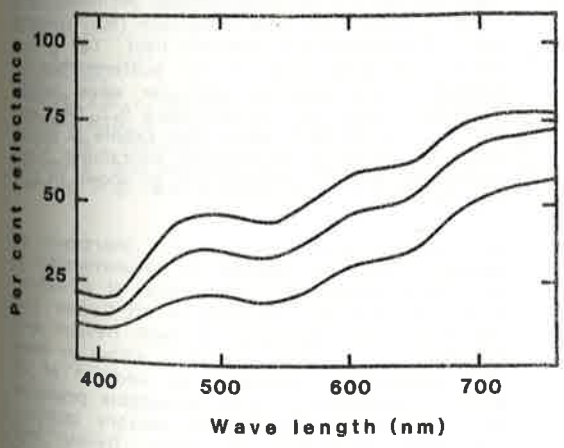

Fig. 2. Reflectance spectra in the visible region 380-760 nm of cut meat loaf surface when blood is added to a basic recipe of 100 kg. The spectra show from top the reflectance when 0, 2 and 5 kg blood are added.

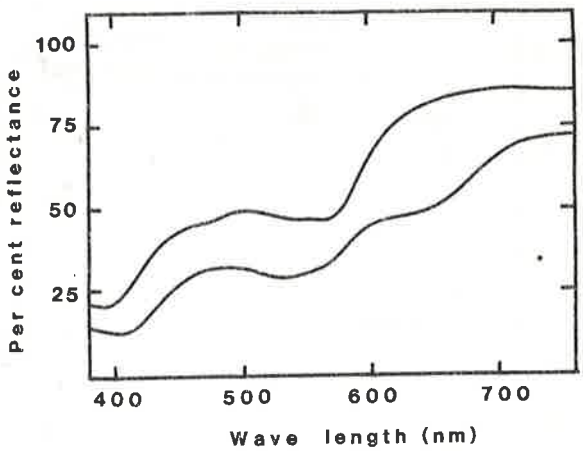

Fig. 3. Reflectance spectra in the visible region 380-760 nm of cut meat loaf with 30 ppm nitrite and 200 ppm ascorbic acid. Top spectrum 0 kg and bottom spectrum 3 kg blood added to the basic recipe of 100 kg.

Results and Discussion

Changes in sensory properties. Fig. 1 shows colour, flavour and texture changes in the product as a function of per cent blood added. For all the sensory variables the main significant changes occured in the range between 2% and 4% blood added. Colour changes, as seen in Fig. 1A, are characterized by a nearly linear increase in darkness in the range of 0-4% blood added. At 2% blood added the total impression of colour is found to differ significantly from the sample with no blood added (0%-sample).

Flavour changes, as seen in Fig. 1B, are clearly found when 3% or more blood is added compared to the 0%-sample. These changes are described by loss of meat flavour, increase in off-flavour and thereby decrease in total impression of flavour. None of the judges did characterize the off-flavour as "blood-taste-like" in the samples, except for those with 4% and 5% blood added. Texture changes, as seen in Fig. 1C, are dominated by increased coarsness when small amounts of blood are added. Further a decrease in hardness is obtained as well as a decrease in the total impression of texture. Juiciness seemed to be unchanged in the range measured. Thus, first of all colour darkness, but also increase in off-flavour and coarsness and decrease in meat flavour and hardness occur when blood is added in meat loaf production. However, an addition of 2% blood is assumed to give acceptable products with respect to the overall sensory quality, i.e. total impression of colour, flavour and texture.

Table I. Visual colour parameters of meat loafs measured by integrating sphere reflectance spectrophotometry. All the values are calculated from Figs. 2 and 3 using C light. The weighted ordinate method (10 nm intervals) was used (Hunter 1975).

Colour system		Reference	2% blood	5% blood	Reference with nitrite	3% blood with nitrite
CIE tristimulus values	X	51.0	40.3	26.3	56.6	37.3
	Y	50.6	39.0	24.7	52.9	35.1
	Z	44.1	33.8	20.8	50.2	31.2
CIE trichromatic	x	0.350	0.356	0.366	0.354	0.360
	y	0.347	0.345	0.344	0.331	0.339
	z	0.303	0.299	0.290	0.314	0.302
CIE	λ_d	581	584	587	591	588
	P_e	0.189	0.203	0.228	0.163	0.197
	P_c	0.261	0.271	0.294	0.205	0.254
CIE	U*	15.6	18.3	20.5	25.4	21.9
	V*	14.6	13.0	11.2	9.83	10.9
	W*	75.4	67.8	55.8	76.8	64.9
CIE 1976	L*	76.5	68.8	56.8	77.8	65.9
	a*	3.80	6.38	8.76	12.0	9.59
	b*	15.3	14.4	13.4	11.4	12.7
CIE 1976	L*	76.5	68.8	56.8	77.8	65.9
	u*	15.7	18.7	20.8	25.7	22.3
	v*	22.1	19.8	17.0	14.9	16.6
Mc Adams	Y	50.6	39.0	24.7	52.9	35.1
	u	0.216	0.222	0.229	0.226	0.227
	v	0.322	0.322	0.323	0.317	0.320
Hunter	L'	71.1	62.5	49.7	72.7	59.2
	a'	5.06	9.33	15.1	16.0	14.7
	b'	18.3	18.7	20.1	13.7	17.2

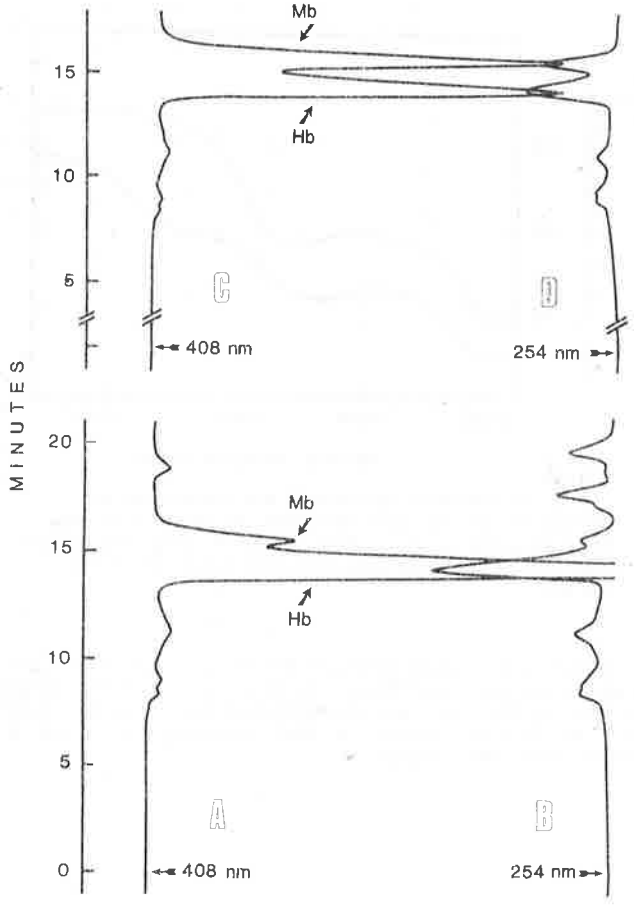

Fig. 4. HPLC chromatograms of hemoglobin (Hb; t_R=14.2 min) and myoglobin (Mb; t_R=15.5 min) of mechanically deboned beef. Ten grams were extracted with 40 ml buffer. Ten µl samples were injected and the wave length detectors were set at 408 nm x 0.2 (A) and 254 nm x 0.16 (B). When the sample is mixed with one part of a solution containing 1 mg Mb/ml the elution profiles are as shown in (C) and (D).

Colour measurements. Figs. 2 and 3 show reflectance spectra of meat loafs produced with blood, and with blood, nitrite and ascorbic acid present compared to a reference. The visual colour parameters in the different colour systems are shown in Table I. When nitrite is present, an increase in lightness is observed and the product becomes more red. This is due to the spectral properties of nitrosoheme, which increase the reflectance in the red region of the spectrum (Fig. 3). Blood decrease the lightness of the product Fig. 2, Fig. 3 Table I) since the increase in heme-pigment decrease the reflectance. The sensory evaluation correspond to the measured colour parameters. When blood is added, a decrease in lightness is always observed (Table I)

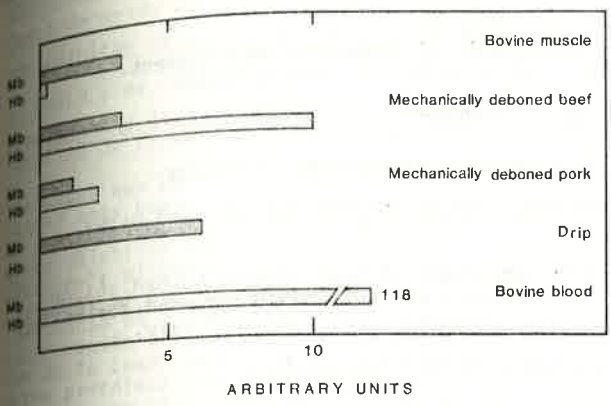

Fig. 5. Relative amounts of hemoglobin (Hb) and myoglobin (Mb) in bovine muscle, mechanically deboned beef and pork, drip obtained from bovine semimembranosus stored 6 days at 3°C, and bovine blood.

which correspond to the sensory evaluation of colour intensity. However, the colour parameters show that λ_d increase when blood is added and there is also a slight increase in spectral purity. Addition of nitrite change λ_d by 10 nm and a red shift of 7 nm is observed with 3% blood as compared to the reference without blood and nitrite. Changes in chroma are seen when comparing the a* and b* values in the CIE 1976 L*, a*, b* system. The a* value increases towards red when blood is added, while a slight decrease is observed in the yellowness of the product. Nitrite increases the red colour of the products as expected. At present a more detailed study of colour and colour changes in sausages when blood is added is performed.

Determination of hemoglobin and myoglobin by high performance liquid chromatography (HPLC).

Since Hb and Mb are the main colour components in meat products, a quantification of these components in the standard raw materials is necessary for a successful colour optimalization of a recipe. Fig. 4 shows how this can be done by HPLC. The absorption at 408 nm (λ band or Soret band) is specific for the heme chromophor in both Hb and Mb, while the presence of other proteins can be detected at 254 nm. The relative values of Hb and Mb shown in Fig. 5 reveal the differences in pigment content among different food samples. Approximately 10% of the pigment in bovine muscle is Hb (see also Warriss and Rhodes, 1977). A rather high amount of Hb is found in mechanically deboned meat and these values varies considerably depending on the optimalization of the deboning process. Drip contains almost only Mb while blood contains only Hb.

Conclusions

The present study shows that small amounts of blood can be used in meat products. The dark colour and off-flavour are the most critical sensory properties with respect to acceptability. Measurement and calculation of visual colour parameters can be used as a practical tool with regard to colour evaluation when new recipes are tested. Since colour is a function of pigment concentration, quantification of Hb and Mb in the standard raw materials is necessary, and this can be performed by HPLC.

Acknowledgement

The technical assistance of Marit Rødbotten, Johanne Margrete Bjørge and Bjørn Sæther is greatly acknowledged.

References

George, J.C., Vallyathan, N.V. and Ronald, K. (1971) Can. J. Zool. 49, 25-30.
Hunter, R.S. (1975) The measurement of appearance. John Wiley & Sons, Inc., London, 348 pages.
Kryvi, H., Flatmark, T. and Totland, G.K. (1981) J. Fish Biol. 18, 331-338.
Slinde, E. and Martens, M. (1982) J. Sci. Fd. Agric. In press.
Warriss, P.D. and Rhodes, D.N. (1977) J. Sci. Fd. Agric. 28, 931-934.