KINETIC ANALYSIS OF CHANGES IN LIGHTNESS ATTRIBUTE OF COLOR DURING THE PROCESSING OF COMMINUTED MEAT PRODUCT

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INTRODUCTION

Comminuted meat processing involves several steps such as comminution/mixing, filling and heating. To gain a comprehensive understanding of the process, extensive knowledge is needed on quality properties of meat raw material and meat batters as well as on the effects of processing stages on them (Smits, 1984). Once such knowledge is obtained, it will serve in optimization of product quality. This knowledge can be incorporated in least-cost formulation and expert system programming.

The color of meat products is one of the most important quality factors, determining the consumer's evaluation and acceptance (MacDougall, 1977). Many reports can be found in the literature on the influence of the processing steps mentioned on the color of meat model systems and specific product preparations (Fox et al., 1967; Reith & Szakaly, 1967; Klettner & Ambrosiadis, 1980; Wirth, 1986; Paneras & Bloukas, 1987). These studies are mostly "end-point" experiments. Approaches providing systematic, scientific evidence or comprehensive kinetic assessment are rare (Jenkins, 1984; MacDougall & Allen, 1984).

Recently a study has started to provide a kinetic description of changes and interrelations between reflection (mainly color measurements) and other physical properties (microstructure, rheology and the state of the muscle proteins) during the processing of comminuted meat. In the first stage of the study an attempt was made to characterize the changes in the visually related color attributes lightness (L^*), hue (H^*) and chroma (C^*) during the processing of a well defined comminuted porcine lean meat (PLM) product (Palombo and Wijngaards, 1989a).

The present paper focusses on the influence of air pressure during chopping on changes in L^* of that PLM product.

MATERIALS AND METHODS

Meat raw Materials

Lean meat from pig leg muscles was trimmed of excess fat and gross connective tissue to a desired constant composition of about 2.5% fat, 21.5% protein and 73% water. The meat was then cut into small pieces. Quantities of 6 kg were packed under vacuum in nylon-polyethylene laminate bags and frozen to -40°C.

Experimental procedure

The day before use 18 kg were thawed in two stages: 10 hours in a water bath at 15°C followed by overnight storage in a water bath of 4°C. The temperature of the meat at the start of the experiment was always about 4°C. 17 kg were placed in a bowl chopper (Laska, model KT 60/3, Linz, Austria) and coarsely chopped for 1 minute at knife and bowl speeds of 2677 and 20 r.p.m. respectively. (These speeds were used through the whole chopping stage.) 2% salt (containing 0.6% sodium nitrite), 0.1% phosphate mixture ("Degens-Latuw" commercial mixture) and 0.05% sodium ascorbate (Sigma, A-7631) were added dry. Chopping was then continued for another 6.5 min divided into 2 min intervals which were interrupted by breaks of 1 to 4 min for temperature measurement and sampling and for obtaining the desired air pressure. The batter was transferred to 100 g cans, placed for 45 min at 20°C, heated according to variable temperature-time combinations, and cooled for 1 h at 0°C (the last 3 steps were done in water baths).

Experimental design
Two levels of pressure during chopping were investigated by means of 7 experiments:
- 4 replicate experiments for chopping under atmospheric air pressure (AAP) and
- 3 replicate experiments for chopping under reduced air pressure (RAP; 0.15 bar).

Each of the experiments was performed on a different day. Within each experiment the following temperature-time regimes were employed: 15°C from 1 min of chopping till 24 h, and 30°C and 40°C up to 3 h.

Color measurement
For every temperature-time combination 2 replicate cans were opened, and the color of their surfaces were measured with a Hunter D25M-9 Tristimulus colorimeter fitted with a D-25M optical sensor. The instrument gave the CIE 1976 L^*, a^* and b^* (CIELAB) values (for a 2° position of the "standard observer" and a "C" type light source) from which the psychometric values lightness (L^*), hue (H^*) and chroma (C^*) were calculated (Wyszecki and Stiles, 1982).

Air determination
% entrapped air was determined using an air tester developed by Vemag (Vemag, Verden, West-Germany). The procedure of determination was as described by Reichert (1988). Five replicate measurements were made for each determination of % entrapped air.

Microstructural examination
At various stages of the process, 6 sample units, 1 cm^3 each, were taken at random. They were mounted on small cork disks, wrapped with aluminum foil and frozen in isopentane cooled with liquid nitrogen. The samples were stored at -80°C until further handling. Shortly before the microscopical examination sections, 6 microns thick, were cut in a Cryostat (type HR, Slee, London, UK) set at -20°C. One representative section was taken from each sample unit for microscopical examination. Employing the dark-ground microscopy (Drury & Wallington, 1980), air bubbles could be easily distinguished from the meat matrix. Three sections from 3 different sample units were used for counting the number of bubbles per field of view (NB) at 100 magnification. For each section, the number of bubbles in each of 15 randomly selected fields of view was counted. The results for the 3 sections were averaged and used for further analysis. The size distribution of the bubbles' diameter (BSD) was determined by the a morphometrical processor (mini-MOP, Kontron, Munich, W. Germany). One similarly prepared section from each of the other 3 sample units was systematically screened to permit at least 100 bubbles to be measured in a section.

Data processing
Iterative fitting of the kinetic data and other statistical analyses were done by using the Genstat statistical package (Genstat manual, 1977).

RESULTS AND DISCUSSION
Results of two typical experiments dealing with changes in lightness of porcine lean meat batter during the various processing stages are shown in figure 1, the one chopped under AAP and the other chopped under RAP. Principally, for both systems we obtained the same pattern of changes: a sharp increase during the chopping stage which reached a maximum value at the end of the comminution. This peak is followed by a gradual decrease during 6-8 hours and from then, the plots approximate a final constant value (plateau).
Comparison of the plots for the two conditions shows that through all the stages lightness values were always higher for chopping in air. Visual assessment of the differences was in agreement with the pattern mentioned. For the sake of clarity, the analysis of the results and further discussion are separated into:
- results obtained during the chopping stage (the increasing phase), and
- results related to changes along the period from end of chopping until 24 h (the decreasing phase).

Chopping stage
To allow quantitative analysis for the changes in L^*, different parameters were defined (Table 1).

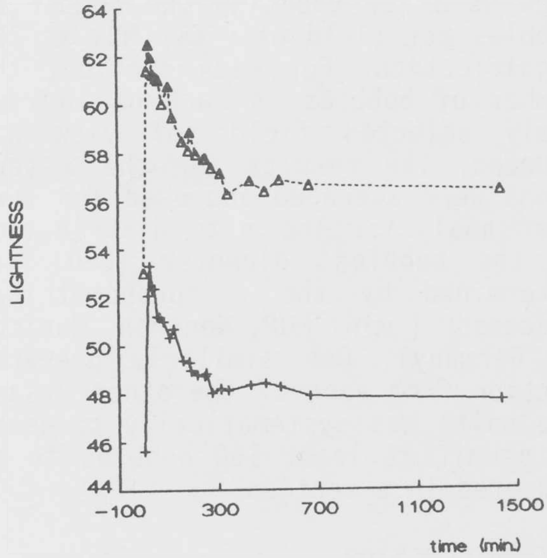

Fig. 1. Changes in lightness of porcine lean meat (PLM) during processing. (Δ-Δ) PLM chopped under atmospheric air pressure (AAP); (+-+) PLM chopped under reduced air pressure (RAP).

Table 1. Summary of t-tests for replicates means of changes in lightness (in L* units) of the porcine lean meat batters during the chopping stage.

Parameter	Chopping under reduced air pressure (a)	Chopping under atmospheric air pressure (b)	S.E.
L^* at 1 min chopping (X)	48.85**	52.94	1.27
L^* at end of chopping (Y)	52.43***	59.17	0.92
Y-X	3.58*	6.23	1.05

* = p<0.05
** = p<0.01
*** = p<0.001
S.E. = standard error
(a) = 3 replicate experiments
(b) = 4 replicate experiments

For both parameters, "X and "Y", we observed significantly higher L* values for chopping in air (Table 1). Determination of the air content of the two meat batters was performed after filling the batters into cans. The following values (in % volume) were obtained: 6.32±0.66 and 1.30±0.24 (Means±S.D.) for the batter chopped in AAP and the batter chopped under RAP respectively. Microscopical observations of the meat surfaces at the end of chopping, revealed a dramatic difference between the two treatments. The batter produced in atmospheric pressure contained a large number of air bubbles mostly in the size range of 10-100 microns. The batter produced under 0.15 bar had a markedly smaller number of bubbles which were mostly bigger than 100 microns (Figure 2). Because of the marked difference in the refractive indices between air and batter matrix, the entrapped air bubbles act as scattering elements. The more encounters caused by refraction of light, the more light is reflected from the surface, making the surface appear lighter (Francis & Clydesdale, 1975). Moreover, opening the lid of the chopper during the chopping intervals exposed the batter chopped under reduced air pressure to an almost 7 times higher pressure. This obviously induced a certain extent of compression which resulted in a more tight and less scattering surface, as was indeed observed in microscopical tests and is supported by the results of the air determination.

End-of-chopping until 24 h
Applying the empirical approach of kinetic mathematical modeling (Labuza, 1983) the following non-linear model was used for describing the changes in L^* during this decreasing phase.

$$L^* = a - b\,[1 - \exp(-ct)]$$

where:
a = initial value (L^* units)
b = extent of decrease (L^* units)
c = rate constant (min^{-1})
t = time (min)

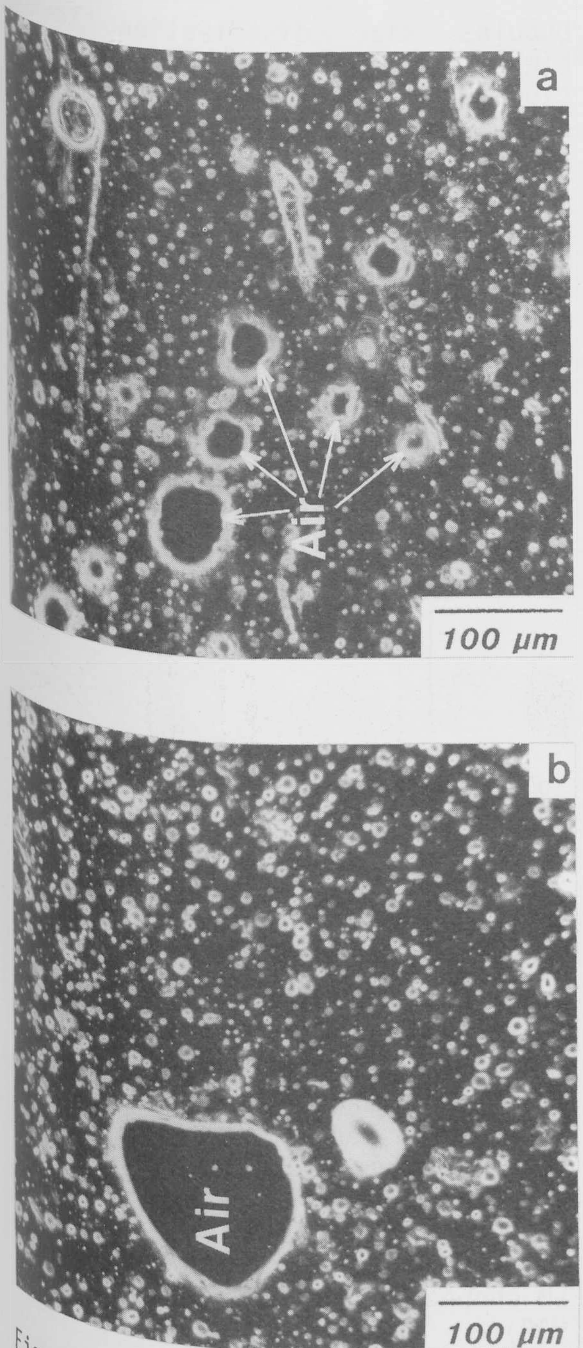

Fig. 2. Microscopical appearance (x100) of porcine lean meat (PLM) batters at the end of chopping. (a) = PLM chopped under atmospheric air pressure (AAP). (b) = PLM chopped under reduced air pressure (RAP).

This model is in fact a modification of a first-order model obtained by addition of an initial value (a) and a final value (a - b). Table 2 presents the analysis of the model parameters. Comparison of the predicted initial L* values (Table 2) with the measured value at end of chopping presented in Table 1 demonstrates the high precision with which the model predict the

Table 2. Summary of t-tests for replicates means of the model parameters for lightness (in L* units) of the porcine lean meat batters.

Parameter	Chopping under reduced air pressure (a)	Chopping under atmospheric air pressure (b)	S.E.
Initial value	53.40***	59.50	0.98
Extent of decrease	5.72**	7.38	0.54
Final value	47.68***	52.12	0.92
Rate constant	0.00725***	0.00445	0.00056

** = p<0.01
*** = p<0.001
S.E. = standard error
(a) = 3 replicate experiments
(b) = 4 replicate experiments

data. Moreover, the variation of the differences between measured and predicted values is comparable with the standard deviation calculated for the replicate measurements.
Chopping in air results in a significantly higher decrease of L* values (b). However, examination of the final values (a-b) reveals that the higher decrease for chopping in air is not sufficient for both systems to reach the same final value.
The rate constant for the decrease in lightness was significantly lower for chopping in air. The same model was used for analysis of the same processing phase for the additional temperatures 30°C and 40°C. Table 3 presents the calculated rate constants for the three temperatures for two experiments representative for the two processing conditions. We can clearly observe, that for air chopping at all three temperatures, the rate constant is lower.
Employing the Arrhenius model for describing the temperature dependence of

the rate constants resulted in an excellent fit , which supports the adequacy of this model for analysis. Calculation of the apparent activation energy (Ea) for both systems revealed that chopping in air yielded a markedly lower value (Table 3).

Table 3. Rate constants (C) and apparent energy of activation for the changes in lightness.

Temperature (°C)	Chopping under reduced air pressure ($Cx10^2$ min^{-1})	Chopping under atmospheric air pressure ($Cx10^2$ min^{-1})
15	1.5	0.8
30	22.8	4.6
40	124.0	14.0
Energy of activation (Kcal/mol)	31.8 (0.999)[a]	19.8 (0.999)[a]

[a] Numbers in brackets are adjusted linear correlation coefficients of Ln(C) vs 1/T

At the end of chopping the PLM batter contains muscle tissue fragments and, as reported here, entrapped air bubbles which are embedded in a viscous sol-like matrix consisting of sarcoplasmic and extracted myofibrillar proteins. Two main processes, able to influence changes in L^*, might be affected by the air pressure during the chopping stage. They are:

- transitions in the entrapped air fraction, and
- changes in the state of the meat pigment Myoglobin (Mb).

<u>Transitions in the entrapped air</u>

Preliminary tests performed on the PLM batter chopped under AAP, showed that, during the decreasing phase entrapped air bubbles disproportionate. Disproportionation is a physical process driven by the Laplace pressure difference over curved bubble surfaces. Gas diffuses from small bubbles to bigger bubbles and a shift in the bubbles size distribution towards higher size classes is observed. Additionally, this process is self accelerating since as bubbles get smaller the driving force increases. Thus, parallel to the shift in bubble size distribution, also the number of bubbles in a unit volume decreases since smaller bubbles will eventually disappear (Prins, 1988). The number of bubbles per field of view (NB) at various processing stages for a PLM batter chopped under AAP is presented in Fig. 3.

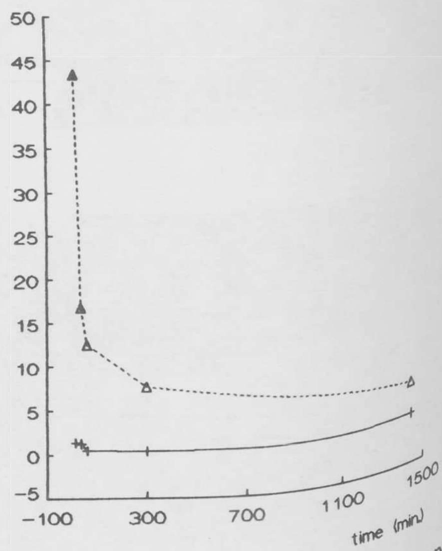

Fig. 3. Number of air bubbles per field of view (NB) of porcine lean meat (PLM) batter at various processing times. (Δ-Δ) PLM chopped under atmospheric air pressure (AAP); (+-+) PLM chopped under reduced air pressure (RAP).

As was also observed through the microscope, from the end of chopping (14 min) until 36 min a steep decrease in NB is obtained. This is followed by a moderate decrease until 5 h. From then until 24 h only minor changes occur. A histogram describing the changes in the size distribution of the entrapped air bubbles' diameter during the decreasing phase in L^*, for the same PLM batter, is shown in Fig. 4. One can clearly observe that, with increasing process time, a marked shift of the BSD towards higher size classes takes place.

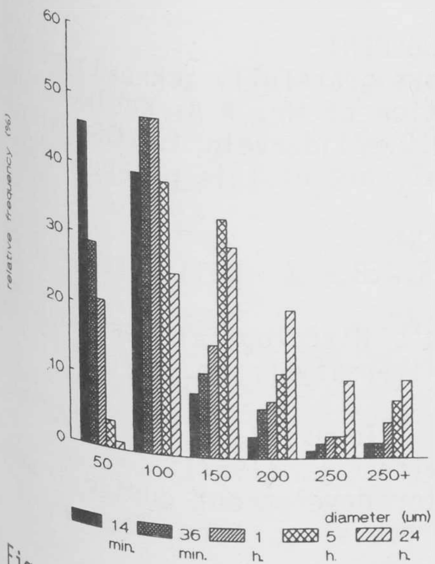

Fig. 4. Size distribution of entrapped air bubbles diameter (BSD), at various processing times, for porcine lean meat (PLM) batter chopped under atmospheric air pressure (AAP).

An attempt to quantify the same parameters for the PLM batter produced under RAP succeeded only for the NB. As can be observed in Fig. 3, an extremely low NB value was obtained for this batter at the end of chopping. From then, a very small decrease commenced and was followed by a near constant value until 24 h. These low NB values hampered the construction of a reliable histogram and detection of shifts in its BSD was not possible.
As stated under the "chopping stage", entrapped air bubbles act as scattering elements. A change in their number and/or a shift in their size distribution is expected to induce changes in the scattering properties of the batter in which they reside. Consequently, a corresponding change in the batter's reflectance properties , and thus in L^*, takes place (Hunter, 1975). Thus, a decrease in NB, together with a shift in BSD towards higher size classes, is expected to cause a decrease in L^*.

<u>Changes in the state of pigmentation</u>
References to the inhibitory effect of entrapped oxygen on the reducing systems active in the conversion of Mb to its nitric oxide form (NOMb) are widespread (Watts et al., 1966; Fox et al., 1967; Wirth, 1986). The formation of the NOMb in the studied PLM batters occurs in the same time interval during which the decreasing phase in L^* takes place. It is characterized by a parallel decrease in hue angle (H^*) which corresponds to a visually detected reddening of the batters surface (Palombo & Wijngaards, 1989a). A comprehensive kinetic analysis of color changes during the processing of comminuted meat was recently reported by Palombo and Wijngaards (1989b). They argued that for the two processing conditions studied changes in rate of this chemical transition in the state of Mb is one of the main factors responsible for the marked differences in the rate constants calculated for the three color attributes L^*, H^* and C^* during this phase.
Hence, the overall effect seen in L^* during the decreasing phase is suggested to be the sum of two simultaneously occurring processes i.e. disproportionation and formation of NOMb. On the basis of the results reported here, it is clear that the extent of changes in bubbles parameters are much more pronounced in the batter produced under AAP. An excess amount of nitrite was added to both of the studied systems at the fist stage of comminution. Thus, at the plateau phase in L^* (see Fig. 1) the total amount of NOMb present in both systems, can be considered the same. In this light, the significantly higher extent of decrease obtained for L^* of this batter can be mainly attributed to the the effect of disproportionation of entrapped air bubbles.
The balance between the relative contributions of the two types of processes, can be used to explain the marked difference between the rate constants obtained for L^* in the two chopping conditions.
In the batter chopped under RAP the partial contribution of disproportionation to the overall decreasing rate in L^* is very small. On the other hand, the rate of NOMb formation is markedly accelerated due to the the lower proportion of entrapped oxygen. Thus the rate of this process will predominantly steer the rate of the observed decrease in L^*.
In the batter chopped under AAP, the

partial contribution of the disproportionation to the overall rate of decrease in L^*, is masked by the decelerating effect induced by the retarded rate of NOMb formation (due to the inhibitive effect of entrapped oxygen). Thus, a lower overall rate of the decrease in L^* results.
Following the same line of reasoning a hypothesis, for the difference in Ea can be offered. Activation energy indicates how sensitive changes in properties or components of food systems respond to temperature changes. A shift in Ea due to specific treatment, might suggest a change in the physicochemical properties of the system. Assume that disproportionation and the reactions responsible for the formation of NOMb have different Ea values and that the Ea for the latter process is the higher one, and assume that during chopping under RAP the process with the higher Ea controls, then a steeper slope will be obtained for the Arrhenius plot as indeed resulted from our analysis.
The exact mechanism steering the observed changes in L^* is obviously a complex one. An attempt to further substantiate and to quantify it, is the subject of our subsequent study.

CONCLUSIONS
During the chopping stage, the marked difference in the absolute values of L^* between the batter produced under AAP and the one produced under RAP, can be explained by a salient difference in their air content.
Evidence for the diproportionation of entrapped air bubbles during the decreasing phase were presented. This process is suggested to induce the difference in the extent of decrease between the two treatments. However, it could not be used to explain the marked difference between their rate constants during this processing stage. The inhibitory effect of oxygen on the rate of reactions responsible for the formation of NOMb could be used to offer an explanation for this phenomenon.
An explanation for the difference in Ea of the two treatments is approached by referring to the balance between the two types of transitions (i.e. disproportionation and reactions responsible for NOMb formation) proposed to steer the overall effect on L^*.

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