KINETIC MODELING OF COLOR STABILITY IN LLAMA (*Lama Glama*) DRIED BLOOD HEMOGLOBIN CONCENTRATE DURING STORAGE

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ABSTRACT

The effect of lactic acid, ascorbic acid and sodium erythorbate at three concentrations (0.5, 1 and 2%) on color parameters (a^* and ΔE^*) of llama spray-dried hemoglobin concentrate was investigated. A kinetic approach was employed to compare the ability of these reducing agents to retain color during spray-drying and storage. Color stability was measured during an 8-week storage period at room temperature. In addition, the macronutrient composition, aminoacid composition and heminic iron content was determined. The addition of 0.5% of lactic acid resulted in the highest retention of redness (a^*) after spray-drying and during storage. The initial rate of a* and ΔE^* change was the slowest and the plateau value for both parameters was the highest. In addition, it was possible to produce different stable reddish tones by applying other concentrations of lactic acid, ascorbic acid and sodium erythorbate. Moreover, this material presented a high nutritional quality in terms of protein content (95.8% d.b.), heminic iron content (3068 ppm) and essential aminoacid composition. Although for the latter, some essential aminoacids were below the recommended standard concentration. To the best of our knowledge, this study is the first in valorizing a side-product, namely blood, from llama meat production chain.

Keywords:

Llama, blood, hemoglobin concentrate, color, kinetic modeling, spray-drying

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1 INTRODUCTION

It is generally acknowledged that color is one of the main properties evaluated by consumers to decide on the quality and freshness of foods. Moreover, this physical property affects the perception of the other sensory characteristics (Clydesdale, 1993) by permitting, in some products, the use of smaller quantities of other additives such as flavorings. Although food colorings are now widely accepted, their use is also controversial. Concerns about food safety, together with current consumer preferences for natural colorants, has led to the substitution of synthetic colorants by natural ones, when it is technologically possible.

There are many natural sources of colorants; however, their commercial potential would be limited by the availability of the raw material. In this sense, blood hemoglobin from slaughtered animals could be an important source of natural red colorant due to the high quantities generated daily. It is important to note that the use of hemoglobin as a food ingredient has the

potential to simultaneously combat iron deficiency, a major nutritional problem in the world today particularly prevalent in children. (Walter et al., 1993; Martínez et al., 1998). However, relatively little relevance has been given to this possibility. Wismer-Pedersen (1988) reviewed the uses of hemoglobin in foods and suggested many different applications but, in any case, the dark color of products containing hemoglobin was an important handicap. The undesirable brown hemoglobin results from oxidation of the red oxyhemoglobin and purple deoxyhemoglobin. Hemoglobin color stability depends on hemoglobin remaining in the reduced ferrous form. Spray-drying has been shown to be a good preservation technology for the red cell fraction, even better than freeze-drying (Toldrá, 2002; Saguer et al., 2003). But high air temperatures, as used in the spray-drying process, enhance ferrous to ferric oxidation. Moreover, oxidation of hemoglobin can also take place during the storage of the spray-dried powder. Since hemoglobin is unstable, its use as a food ingredient depends on its stabilization. There is literature about patents and scientific publications about it but fewer works involve the use of spray-dried or freeze-dried animal blood as a colorant (Francis, 1986).

Recently, advances in spray-drying combined with ingredient/formulation technology have led to the development of some microencapsulated natural pigments (Downham and Collins, 1997). However, we have focused our research on trying to find a suitable hemoglobin color-fixing or protecting agent such as lactic acid, ascorbic acid and sodium erythorbate.

2 MATERIALS AND METHODS

2.1. Blood sampling

Blood was collected under hygienic conditions from llamas aging between 2 and 3 years. The animals were grown in the region of Pasco, Peru. While llamas were slaughtered, blood was sampled by introducing a sterile needle directly to the jugular vein. The sample was conducted to a sterile bag which contained a 3% citrate sodium solution as anti-coagulant (9 parts of blood and 1 part of anti-coagulant). Consequently, the transport of blood in a liquid state was possible to further separate the cell fraction with relative ease. Afterwards, temperature was immediately decrease to the final storage value of 4°C.

2.2 Concentrated hemoglobin production

Stored blood was pooled and centrifuged (2528g for 15 minutes) at 5°C to separate the cell fraction from the plasma by decantation of the latter. The cell fraction was hemolyzed to release the hemoglobin pigment. Hemolysis was performed by diluting the cell fraction in water (3:1) and then agitated for one hour. This provoked erythrocyte membrane breakage, thus releasing the hemoglobin. Subsequently, a second centrifugation step (2528g for 30 min) permitted the elimination of membrane residues and other heavy cell materials to obtain the solubilized hemoglobin concentrate.

2.3 Addition of reducing agents

After obtaining the hemoglobin concentrate, it was divided in aliquots. Three types of reducing agents (ascorbic acid, lactic acid and sodium erythorbate) were added in three concentrations (0.5, 1 and 2% w/w) to stabilize the hemoglobin molecule. A control sample (no reducing

agent) was also considered. Next, hemoglobin concentrate solution was spray-dried (inlet and outlet temperature 150 and 80°C, respectively). Samples were packed in aluminum bags and stored for 55 days at room temperature.

2.4 Dried hemoglobin concentrate macronutrient composition

Dried hemoglobin concentrate was analyzed in terms of chemical composition. Humidity was determined by the oven method (AOAC 930.15), protein by Kjeldahl method (AOAC 2001.11), fat by Soxhlet method (AOAC 923.03), ash by gravimetry method (AOAC 923.03) and carbohydrates by difference with the other macronutrients.

2.5 Amino acid profile

The total amino acid content in dried hemoglobin concentrate was performed by HPLC-DAD. A previous hydrolysis step is necessary to release the constituent amino acids. Dried hemoglobin concentrate (0.1 g) was mixed with 17 mL of a solution composed by 6M HCl, % phenol and 1% Na2SO3, and further diluted to exactly 25 mL. This mixture was incubated at 105°C for 24 hours. Samples pH values were adjusted to 2.2 using 6N HCl. The hydrolyzed sample was filtered using a 0.45 µm low protein binding hydrophilic PTFE membrane. A volume of 20 µL was injected to the HPLC system (Agilent Technologies, Germany). In the injector of the HPLC, primary and secondary amino acids were derivatized by o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), respectively. The derivatized amino acids were separated on a Zorbax Eclipse AAA Rapid Resolution column (4.6 × 150 mm, Agilent Technologies) (Kerkaert et al, 2011).

2.6 Heminic iron content

Heminic iron content of dried hemoglobin concentrate was determined by ICP-MS. First, heminic iron was extracted. Dried hemoglobin concentrate (0.1 g) was weighed in a centrifuge tube where 10 mL of acetone:water:hydrochloric acid (80:10:10 v/v) was added. This mixture was vortexed, maintained in the dark for 1 hour and vortexed again. Afterwards, samples were centrifuged (885 g for 10 min). An aliquot (0.25 mL) of the supernatant was diluted in a HNO_3 solution (4.75 mL). The diluted sample was analyzed by ICP-MS (Cross et al, 2012).

2.7 Instrumental color

Dried hemoglobin concentrate color was measured using the CIE $L^*a^*b^*$ color space were L^* represents the lightness (0, dark – 100, white), $+a^*$ the redness, $-a^*$ the greenness, $+b^*$ the yellowness and $-b^*$ the blueness. The instrument utilized for this purpose was a colorimeter Minolta CR-300 (Metrolab International). The duration of the color stability study was 8 weeks. The color was measured for samples treated with different reducing agents immediately after the spray drying (day 0) and during the following 55 days of storage (day 1, 2, 3, 4, 5, 6, 13, 20, 27, 34, 41, 48 and 55).

The total color difference (ΔE^*) was calculated based on difference between L^*, a^* and b^* values of treated samples and L^*_0, a^*_0 and b^*_0 corresponding to the control sample. The following equation (1) was employed to calculate ΔE^*:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

2.8 Statistical analysis

The behavior of color parameters was modeled using a first order fractional conversion equation (2). In this equation, X is the estimate of the color response evaluated as a function of storage time t (days), X_0 the estimated parameter at the beginning of the storage (after spray-drying), X_f the estimated *plateau* parameter and k_t the estimated reaction rate constant (days^{-1}) at storage temperature.

$$X = X_f + (X_0 - X_f) \exp(-k_T t) \quad (2)$$

Only color responses a* and ΔE^* were modeled with equation (2) since these were the ones showing a clear trend over the storage time.

3 RESULTS AND DISCUSSION

3.1. Dried hemoglobin concentrate macronutrient composition

Blood is an excellent source of nutrients not only in terms of content but also because of its high nutrient bioavailability (Parés *et al*., 2011). With respect to the present research, the composition of dried hemoglobin concentrate was determined. Results of these determinations are summarized in Table 1.

After spray drying, water content was reduced to 5.05%. This value is slightly lower than the monolayer water content of dried hemoglobin concentrate reported for porcine (5.9%) (Saguer *et al*, 2003). According to the water activity theory, dried food stability is reached below the monolayer water content (Labuza *et al*, 1980). On the one side, with respect of carbohydrates and fat, the concentration in llama hemoglobin concentrate is much lower compared to the concentrations in blood of pork, chicken and duck. On the other side, protein content in the hemoglobin concentrate was found in higher concentration compared to pork, cow and other meat production animals (Surapukdee y Narunatsopanon, 2017) (Gorbatov, 2008). These results are logic since plasma was separated from the hemoglobin fraction. The blood plasma contains the majority carbohydrates and lipids because it is the transport media for these nutrients while proteins are more concentrated in the hemoglobin fraction.

3.2 Heminic iron content

Iron content of the blood cell fraction obtained in this study corresponds to 100% of heminic iron, which constitutes the iron fraction with higher bioavailability (Lincan, 2003). Heminic iron content in the blood hemoglobin concentrate (3068 ppm) was much higher compared to pork, chicken and duck blood (1490.14, 1816.62 y 1803.06 ppm, respectively) (Surapukdee y Narunatsopanon, 2017). This difference is partially due to the dilution effect of other constituents in plasma fraction of blood. Nevertheless, it has been reported that American camelids hemoglobin content in blood (and consequently heminic iron content) is higher compared to other meat production species (Quispe, 2011). Therefore, this hemoglobin concentrate has a potential as an excellent source for high-availability iron.

Table 1. Spray Dried Cell Fraction Composition

Component	**Concentration** (g/100g dry matter)
Macronutrient composition	
Moisture	5.05 +/- 0.01
Carbohydrate	0.11 +/- 0.01
Ash	3.97 +/- 0.07
Fat	0.16 +/- 0.04
Protein	95.81 +/- 1.71
Iron Content	
Iron (ppm)	3068 +/- 10

3.3. Aminoacid Profile

The aminoacid profile of dried hemoglobin concentrate is presented in Table 2. An elevated concentration of essential aminoacids was quantified with respect to the standard established by FAO/OMS/UNU (1985), except for isoleucine, tryptophan and sulfured aminoacids (methionine and cystine). These results are in line with the findings of other authors with respect of the deficiency in blood of the latter aminoacids (Parés et al, 2011). Nonetheless, an important concentration of lysine was determined in llama dried hemoglobin concentrate. This is an interesting fact since this material might be used as an ingredient mixed with lysine-deficient sources (e.g. cereals).

3.4. Color Stability Study

The instrumental color of dried hemoglobin concentrate was measured immediately after spray-drying and over a storage period of 55 days to investigate the effect of different reducing agents on its color stability. On the one hand, as mentioned before, L* and b* did not show significant changes nor a clear trend over storage time. Therefore, these two parameters were not included as such for the data modeling. On the other hand, a* and ΔE* showed first order kinetics trends.

In Figure 1, the evolution of a* over storage time is showed for 3 reducing agents at 3 different concentrations added before spray-drying. It can be observed that there is a fast decay of a* within the first week for all treatments. Then this redness decay levels to a plateau value which is stable for the rest of the storage. A similar behavior was reported by other authors who employed reducing agents such as ascorbic acid, glucose and dextrin (Toldrá et al, 2000) or nicotinic acid and nicotinamide (Saguer et al, 2003). Regarding the protective effect against drying, samples treated with 0.5% of sodium eritorbate, 0.5 and 1% of lactic acid were the ones which best retained redness after spray drying (intercepts with y axis). With respect to the redness retention over the storage time, the addition of 0.5% of lactic acid reached the

maximum plateau value (a* value of ~18). As a consequence, this concentration of lactic acid showed the best protective effect during storage.

Table 2. Dried hemoglobin concentrate Aminoacid Profile (mg/g protein) Versus Pattern

	Aminoacid	Llama hemoglobin concentrate	Pork whole blood	Aminoacid pattern proposed for children (1-3 years old)
Essential Aminoacids	Leucine	138	132	66
	Lysine	99	97	58
	Valine	85	87	35
	Histidine	81	88	18
	Threonine	46	48	34
	Isoleucine	17	9	28
	Tryptophan	8	15	11
	Methionine + Cystine	12	24	25
	Phenylalanine + Tyrosine	109	121	63
Non Essential Aminoacids	Aspartic acid	126	-	-
	Alanine	82	76	-
	Arginine	47	-	-
	Glutamic acid	59	-	-
	Glycine	49	-	-
	Proline	35	-	-
	Serine	55	-	-

The reduced form of iron (Fe^{+2}) is the only one able to bind oxygen and form oxyhemoglobin (bright red). However, conditions of excessive acidity or alkalinity affect the affinity of hemoglobin with oxygen, favoring the oxidation of iron (Fe^{+3}) which results in the formation of methemoglobin (brown). In the same way, slight alkaline conditions help to fix oxygen (García et al., 2014). This explains the direct effect of each type of reducing agent used and their respective concentrations in the affinity of hemoglobin for oxygen, generating a deviation in its dissociation curve and therefore in its coloration.

The total color difference (ΔE*) between the untreated sample (control) and the samples treated with reducing agents were calculated with Equation 1. The change in the value of ΔE * was clearly dependent on the storage time and only due to the decrease in the value of a *. Cserhalmi et al. (2006) reports that ΔE* can be used as an indicator to verify if color changes can be

perceived by humans. These authors reported that when ΔE * exceeds the value of 3.0, the difference in color can be quite visible to consumers. Figure 2 shows that the ΔE * value of 3.0 is exceeded very fast on the first day of storage for 1% ascorbic acid (AA 1%) and on the third day for 1% and 0.5% lactic acid (AL 1% and AL 0.5%). In fact, color changes can be noticed, a sample can continue to be accepted by consumers or valid for technological purposes. Similar to the redness, the addition of 0.5% lactic acid leads to the smallest change in ΔE*, which means that color is better retained by this reducing agent.

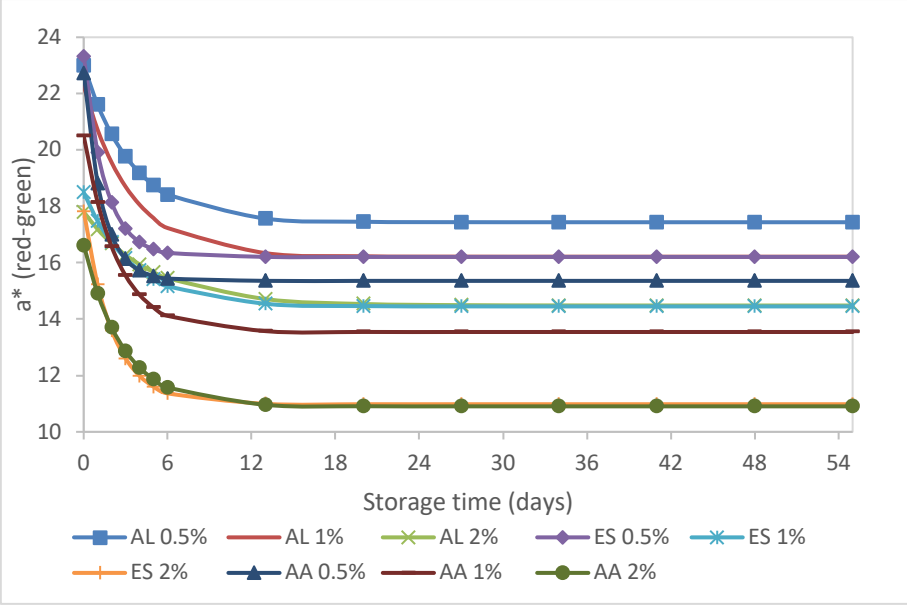

Figure 1. Effect of different reducing agents on redness (a*) retention during storage at room temperature.

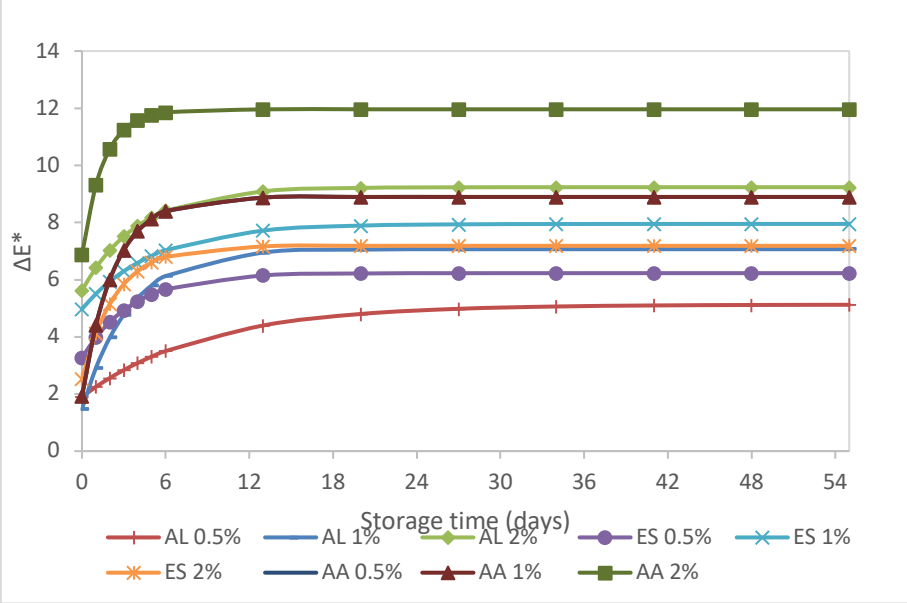

Fig. 2. Effect of different reducing agents on total color change (ΔE*) during storage at room temperature.

4 CONCLUSIONS

It was possible to obtain scales of reddish tones (a*) as a standardized and stable sensory attribute over time of the dehydrated cellular fraction of flame blood with the use of different types of reducing agents and at different concentrations for their application in different food bases. The application of 0.5% of lactic acid seems to retain the redness and retard loss of color during storage. In addition, the high nutritional value of this side-product was demonstrated. Although the dehydrated cell fraction is deficient in methionine and sulfur amino acids; It has a high content of lysine, which is usually limiting in cereals. Therefore, supplementing the diet with a small percentage of dehydrated cell fraction is interesting from the point of view of added value and totally or partially replace other high-quality protein ingredients.

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