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Analytical Methods

Equations for spectrophotometric determination of relative concentrations of myoglobin derivatives in aqueous tuna meat extracts

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ABSTRACT

The percentage of metmyoglobin (%metMb) in aqueous meat extracts of bigeye and bluefin tuna and beef samples were estimated using previously reported equations derived from the absorption spectra of horse Mb. The results demonstrate that in an aqueous extract, the difference in %metMb estimated by the different equations was negligible for beef samples. Conversely, in an aqueous tuna extract, different %metMb values were obtained with the different equations. The discrepancy in the tuna sample results might be due to differences in absorption spectra for horse and tuna Mb. Therefore, a new set of equations derived from the absorption spectra of bigeye tuna Mb, reported by Matsuura and Hashimoto (1955), was established. The accuracy of the proposed equations was compared with the cyanmetmyoglobin (cyanmetMb) method. The results show that the total Mb concentrations estimated by our proposed equations were in good agreement with the results obtained by the conventional cyanmetMb method (R^2 = 0.984). Therefore, the new set of proposed equations is valid for the spectrophotometric determination of the relative proportions of Mb derivatives and total Mb concentration in aqueous tuna meat extracts.

1. Introduction

The red colour of tuna meat is an important factor used in the evaluation of meat quality, and strongly influences the consumer's purchasing decision. It is known that the red colour of meat depends upon the concentration of myoglobin (Mb) and its derivatives (Faustman, Yin, & Nadeau, 1992; Hood, 1980). During storage, the desired red tuna meat undergoes discolouration and develops an unappealing brown colour, which results from the oxidation of ferrous Mb (deoxymyoglobin, deoMb and oxymyoglobin, oxyMb) derivatives to ferric metmyoglobin (metMb) (Bito, 1965, 1976). With the recent rise in the demand for high-quality fresh and frozen fish in the world market (Catarci, 2004), an increasing amount of research is focusing on the colour changes or Mb oxidation, not only in tuna meat, but also in many other kinds of red fish meats (Benjakul & Bauer, 2001; Chen, 2003; Chaijan, Benjakul, Visessanguan, Lee, & Faustman, 2006; Chaijan, Benjakul, Visessanguan, & Faustman, 2005, 2007; Viriyarattanasak, Matsukawa, Hamada-Sato, Watanabe, & Suzuki, 2008; Viriyarattanasak, Watanabe, & Suzuki, 2007). To assess the oxidation of Mb, many studies have employed visible spectrophotometry and Mb oxidation was commonly reported in terms of the percentage of metMb (%metMb). In visible spectrophotometry, pigment extraction is done prior to absorbance measurement, and then the relative concentrations of Mb derivatives is estimated from the measured absorption spectra using equations derived from the application of Lambert–Beer Law. Almost all of the previously reported equations were derived from absorbance coefficients of horse Mb (Broumand, Ball, & Stier, 1958; Krzywicki, 1979, 1982; Tang, Faustman, & Hoagland, 2004). Since horse and beef Mb are considered to have the same spectrometric profiles (Broumand et al., 1958; Stewart, Hutchins, Zipser, & Watts, 1965; Wolfe, Watts, & Brown, 1978), it is suggested that the relative concentrations of Mb derivatives for aqueous beef extract should be determined by using the equation derived from the absorption spectra of horse Mb. Nevertheless, a suitable equation derived from the absorption spectra of tuna or other fish Mb has not been recently reported.

There are differences in some chemical and physical properties of fish and mammalian Mb, such as the primary structure (Ueki & Ochiai, 2004; Watts, Rice, & Brown, 1980) and absorption spectra (Amano & Tsuyuki, 1975; Brown, Martinez, Johnstone, & Olcott, 1962; Matsuura & Hashimoto, 1955). Matsuura and Hashimoto (1955) reported that for tuna and horse, the absorption spectra of metMb and deoMb in the visible region are similar, but those of oxyMb are quite different. Fig. 1 shows peak of the absorption

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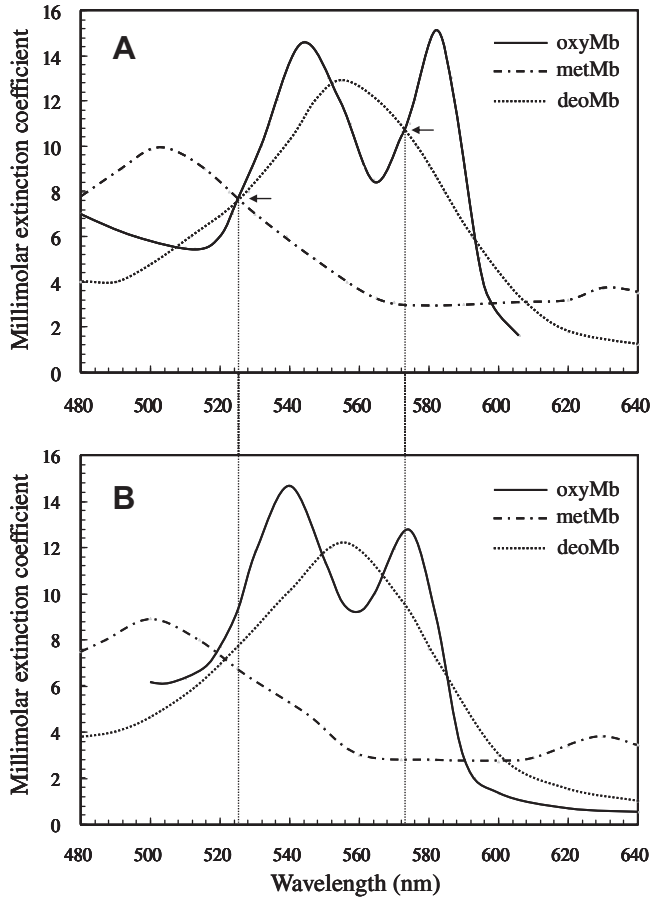

Fig. 1. Absorption spectra of Mb derivatives for (A) horse (Bowen, 1949) and (B) bigeye tuna (Matsuura & Hashimoto, 1955). The arrows show the isobestic point for horse Mb at 525 nm, where the extinction coefficient of three Mb derivatives are equivalent, and at 572 nm, where the oxyMb and deoMb have identical extinction coefficients.

spectra for tuna oxyMb at a shorter wavelength, compared to that of horse oxyMb. Furthermore, the extinction coefficient at the β-maximum (ca. 540 nm) of tuna oxyMb is higher than that at the α-maximum (ca. 580 nm), while a contrasting result is shown for horse oxyMb. Fig. 1 also shows the difference in wavelengths at isobestic points (intersections) between horse and tuna Mb. For example, the oxyMb and deoMb from horse have identical extinction coefficients at 572 nm. However, in tuna this isobestic point is shifted several nanometres to a shorter wavelength. Moreover, in tuna there is no isobestic point at 525 nm, where the extinction coefficients of the three Mb derivatives are equivalent. The isobestic point at 525 nm is characteristically observed in the absorption of horse Mb (Bowen, 1949; Broumand et al., 1958). The wavelengths at the absorption maxima and the isobestic points are commonly selected in the derivation of equations for the estimation of the relative concentrations of Mb derivatives. Therefore, the differences in these parameters between horse and tuna Mb may result in the over- or under-estimation of values when the relative concentrations of tuna Mb derivatives are estimated from equations derived from horse Mb and vice versa (Broumand et al., 1958). Consequently, this may lead to erroneous results and inaccurate interpretations.

Matsuura and Hashimoto (1955) reported that all derivatives of Mb prepared from bigeye and bluefin tuna have almost the same absorption maxima and minima characteristics. Brown et al. (1962) also found that the wavelengths and the absorbances at the absorption maxima of metMb and deoMb from albacore, bluefin, and yellowfin tuna were almost identical. These data suggest that in all Mb derivatives, the absorption spectra from the different species of tuna seem to be identical. Therefore, the absorption spectra data of bigeye tuna Mb, which was reported to be entirely in the visible region for the three Mb redox forms by Matsuura and Hashimoto (1955), was chosen to establish a set of equations for the estimation of the relative concentrations of Mb derivatives in aqueous tuna extracts in the present study.

The objective of this study is to demonstrate the inappropriate determination of the relative concentrations of Mb derivatives in aqueous extracts of tuna samples by using the previously reported equations established from the absorption spectra of horse Mb. Furthermore, a new set of equations derived from the absorption spectra of bigeye tuna Mb, reported by Matsuura and Hashimoto (1955), is proposed. Additionally, we compared total Mb concentrations calculated using a new set of proposed equations and those determined by the cyanmetmyoglobin method (Warriss, 1979).

2. Materials and methods

2.1. Meat sample preparation

Fresh (unfrozen) specimens of bigeye (*Thunnus obesus*) and bluefin tuna (*Thunnus orientalis*), ground beef, and cut beef meat (0.5 × 5 × 8 cm) were purchased from a local retailer. Typically, in Tokyo, fresh fish and beef are transported to a local retailer in the morning, and then they are immediately filleted or ground prior to selling on the same day. Additionally, each tuna specimen used in this study was sold for consumption as a raw fish. It was suggested that fish consumed raw should not contain the %metMb more than 30% (Takai, 2000). The bigeye and bluefin tuna samples were taken from ordinary dorsal muscle. In this study, there were 5 specimens of bigeye tuna, 1 of bluefin tuna, 3 of ground beef, and 1

Table 1
Equations for spectrophotometric determination of the relative concentrations of myoglobin (Mb) derivatives.

	Equations	Reference
(1)	$\%metMb = (1.395-[(A_{572}-A_{700}/(A_{525}-A_{700})]) \times 100$	(Krzywicki 1979; Chen 2003)
(2)	$\%metMb = (1.395-[(A_{572}-A_{730})/(A_{525}-A_{730})]) \times 100$	(Fernandez-Lopez et al., 2003; Krzywicki 1979)
(3)	$\%metMb = (1.395-[(A_{572}-A_{730} \times 1.45)/(A_{525}-A_{730} \times 1.73)]) \times 100$	(Krzywicki 1979; Trout 1990)
(4)	$\%metMb = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100$ $\%deoMb = (0.369R_1 + 1.140R_2-0.941R_3 + 0.015) \times 100$ $\%oxyMb = (0.882R_1-1.267R_2 + 0.809R_3-0.361) \times 100$ $R_1 = A_{572}/A_{525}\ R_2 = A_{565}/A_{525}\ R_3 = A_{545}/A_{525}$	(Krzywicki 1982)
(5)	$\%metMb = (-0.159R_1-0.085R_2 + 1.262R_3-0.520) \times 100$ $\%deoMb = (-0.543R_1 + 1.594R_2 + 0.552R_3-1.329) \times 100$ $\%oxyMb = (0.722R_1-1.432R_2-1.659R_3 + 2.599) \times 100$ $R_1 = A_{582}/A_{525}\ R_2 = A_{557}/A_{525}\ R_3 = A_{503}/A_{525}$	(Tang et al., 2004)