Med & Health Dec 2017; 12(2): 156-169 https://doi.org/10.17576/MH.2017.1202.01

REVIEW ARTICLE

Aspartic Acid Racemization Method for Age Estimation in Human Tissues: A Review

GUMPANGSETH T[1], MAHAKKANUKRAUH P[1,2,3]

[1]Department of Anatomy, [2]Forensic Osteology Research Center, Faculty of Medicine, [3]Excellence Center in Osteology Research and Training Center (ORTC), Chiang Mai University, 50200, Thailand.

ABSTRAK

Peraseman asid amino adalah tindakbalas kimia yang bergantung kepada perubahan sifat protein dalam organisma. Terdapat perubahan dalam kepekatan L- dan D-asid amino semasa hidup dan selepas kematian. Perubahan ini berhubung kait dengan proses penuaan. Asid aspartik (Asp) adalah asid amino tidak perlu yang biasa digunakan pada peraseman kerana ia terkumpul dengan kadar yang cepat kepada asid D-asid aspartik berbanding dengan asid amino yang lain. Oleh itu, proses penuaan berhubung kait dengan penukaran antara asid amino yang merupakan satu peralatan yang boleh digunakan untuk anggaran usia dalam sains forensik. Peraseman asid aspartik berlaku dalam banyak struktur manusia seperti gigi, tulang, rawan artikular, cakera intervertebra, ligamen kuning, kulit, parenkima paru-paru, aorta, dan kanta mata. Tambahan pula, hubungan antara peraseman asid aspartik dan umur boleh digunakan untuk penyiasatan perolehan protein dan penyakit. Terdapat pelbagai kaedah penyediaan asid aspartik yang menyebabkan keputusan yang berbeza diperolehi. Ulasan ini membincangkan kaedah peraseman asid aspartik, faktor-faktor yang mempengaruhi peraseman, aplikasi peraseman dalam penganggaran umur dan beberapa kaedah penyediaan berdasarkan peraseman dalam gigi dan tulang.

Kata kunci: penganggaran umur, asid aspartik, tulang, sains forensik, peraseman, gigi

ABSTRACT

The amino acid racemization is a chemical reaction process that depends on the property change of proteins in the organism. There is change in the concentration of L-and D-amino acids in during life and after death, as well. This change leads to the relationship with aging process. Aspartic acid (Asp) is non-essential amino

Address for correspondence and reprint requests: Pasuk Mahakkanukrauh. Excellence Center in Osteology Research and Training Center (ORTC), Chiang Mai University, Chiang Mai, 50200, Thailand. Tel: +66-53-949-474 Fax: +66-53-945-304 E-mail: pasuk034@gmail.com

acid which is commonly utilized on racemization because it has most rapidly accumulated of D-aspartic acids of all amino acids. Therefore, aging is related to the interconversion of amino acids, which is a useful tool for age estimation in forensic science. Aspartic acid racemization can occur in many human structures e.g. teeth, bone, articular cartilage, intervertebral disc, yellow ligament, skin, lung parenchyma, aorta, and eye-lens. Additionally, the relationship between aspartic acid racemization and age can be used for investigation of protein turnover and pathological diseases. There are different aspartic acid preparation methods which obtain different results. In this review, we describe aspartic acid racemization method, several factors of racemization, application of racemization for age estimation including several preparation methods based on racemization in both teeth and bones.

Keywords: age estimation, aspartic acid, bone, forensic science, racemization, teeth

INTRODUCTION

Human skeletal remains are commonly used in forensic anthropology to identify of unknown individuals (Konigsberg et al. 2008) particularly in cases where the remains are presented in the form of fragmentation, burning or severe damage from other causes such as mass disasters, fires or crimes (Owsley et al. 1985). The data of skeletal remains have established a biological profile that can be compared to unidentified individuals. The biological profile consists of sex, age, ancestry and stature. These data are very useful variables for narrowing the range of identification (Blau & Briggs 2011; Cattaneo 2007). Moreover, the data from finger prints, dental data and DNA analysis provide the most accuracy for specific individuals. But these methods take a long time to analyze data and are expensive (Carolan et al. 1997) or few countries lack DNA database. Thus, the information from biological profile is first usually applied in forensic context for primary identification (Qudsia et al. 2014).

The various methods for age estimation are based on the assessment of developmental and degenerative changes in the skeletons such as macroscopic morphological studies (Albert 1998; Brooks 1955), histomorphometry method (Inthasan & Mahakkanukrauh 2017; Kerley 1965), radiological assessment (Martin et al. 1981), and biochemical method (Helfman & Bada 1975; Helfman & Bada 1976). There are several biochemical processes in protein which is related to determining age such as oxidation, isomerization and racemization process (Balin & Allen 1989; Stadtman 1988). Racemization of amino acid method has been generally used in forensic sciences because this analysis process is more objective and accuracy (Ogino et al. 1985; Ohtani 1995b; Ohtani & Yamamoto 2010).

AMINO ACID

Amino acid is the building block of proteins in which one amino acid connects to another one to become chains of polypeptide. Many polypeptides are connected together to protein. Generally, chemical form of amino acid is composed of an amino group (NH_3), a carboxyl group (COOH), a hydrogen atom (H), and R group or side chain which relies on the type of amino acids. The four different groups or atom bond to an alpha carbon atom in the centre (Johnson & Miller 1997; McCudden & Kraus 2006). Amino acids combine with other molecules by peptide bond which forms between amino group of one amino acid and the carboxyl end. Amino acids within organism proteins have important role in many systems in the body such as structure of cells, transportation and storage, formation of enzymes or antibodies (Robins et al. 2001). Amino acids commonly present in two forms which are L-(levorotary) and D-(dextrorotary) forms. These forms are enantiomers that mean both L- and D-forms are mirror image of each other and is not superimposed like the right and left hands. L-amino acids use in protein synthesis (Poinar et al. 1996). The chemical property of two forms is not different (Bada 1982). Amino acids within the proteins initially form in the L-form and during aging the L- form of amino acids will be gradually converted to D-form (Iida et al. 2001). There is accumulation of D-form increased with age. Therefore, interconversion of both forms amino acids involves organism age (Holtkötter 2011; Johnson & Miller 1997). This process is called amino acid racemization.

AMINO ACID RACEMIZATION

Racemization is the reversible first-order reaction of amino acids that is the transformation of L- and D-form amino acids. This reaction takes place in the tissues with low metabolic turnover rate at body temperature in mammalian (Masters et al. 1978; Ohtani et al. 2002). This process is depended on temperature, time, pH (Fisher et al. 1986). Age estimation from low metabolism tissues provide more precise than high metabolism tissues because in tissues with high metabolic rate have continually form L-amino acids. So, there is not formation of D-amino acids. The change D/L ratio of amino acid during aging can be used to estimate the death time of the organism. The low protein turnover tissue which is usually used for amino acid racemization is teeth and racemization rate is approximately 0.1% per year in the low metabolism tissue like dentine (Csapo et al. 1994). Moreover, racemization can be occurred in various structures such as the bones, white matter of brain, artery and eye-lens (Dobberstein et al. 2010; Griffin et al. 2008a; Johnson & Miller 1997). The previous study (Hare & Abelson 1968) examined amino acids in fossils shell. They reported that D-amino acids were obtained from transformation of L-amino acid and the amount of D-amino acids was continually increased with age. Furthermore, there was higher the

D/L ratio of amino acids in older fossil shells. Then, there were application of amino acid racemization in fossils to estimated age by measurement the D/L ratio of amino acids (Hare & Mitterer 1969). Consequently, amino acid racemization is widely applied for age estimation in many tissues which comprise proteins. Furthermore, the relationship between amino acids racemization and age can be used for investigation of lifetime of protein ageing (Helfman et al. 1977) and pathological diseases in older individuals (Powell et al. 1992; Shapiro et al. 1991).

ASPARTIC ACID RACEMIZATION

There are about 20 general amino acids in human body that divided into essential and non-essential amino acids (Ouellette & Rawn 2014). Aspartic acid (Asp) is non-essential amino acids in the body which commonly used for racemization in age estimation. Racemization of most amino acids is slow process but the accumulation of D-aspartic acids is most rapidly of all stable amino acids in the bones and other tissues (Robins et al. 2001; Yekkala et al. 2006). Aspartic acid racemization has rate constants at neutral pH (Poinar et al. 2006) and can occur throughout lifetime and after death, as well (Yekkala et al. 2006). There was a report about racemization rate of many amino acids in fossil and it was found that racemization rate of aspartic acid was highest when compared to other amino acids (Ohtani et al. 2002). Aspartic acid has weak peptide bond in protein chain so, it has rapid racemization rate (Goodfriend et al. 1992). Rate of racemization is one of the important factors for accuracy of age estimation. Since aspartic acid has the highest rate of racemization, there is adequate amount of D-aspartic acids for amino acid analysis. Thus, aspartic acid is the most suitable indicator of age determination (Ohtani et al. 2002).

Racemization of aspartic acid in polypeptide chain relates a cyclic succinimide intermediate or succinyl residue (Asu) (Van Duin & Collins 1998). Hydrolysis of aspartic residue will be produced a succinmide intermediate (Robins et al. 2001) via the process is called nucleophilic attack. Several of the sites of suucinimide intermediate formation usually occur in the terminal regions of proteins. The process initiate from the nitrogen of the carbon terminal peptide bond attract to the carbon molecule of carboxylic acid group creating the conformation of unstable sucinimide ring or called cyclization. This succinimide ring is short lived structure so, it has rapid hydrolysis. Because of this metastable property of succinimide ring, the ring is susceptibility. Thus, the ring structure can be transformed from L-aspartic acid into D-aspartic acid rapidly. In addition, formation of D-aspartic acids also involves asparagines (Asn) by deamidation process (McCudden & Kraus 2006; Ritz-Timme & Collins 2002). Additionally, the racemization rate of aspartic acid in peptide bond is due to reaction of succinimide intermediate formation (Geiger & Clarke 1987; Radkiewicz et al. 1996).

FACTORS TO RACEMIZATION

Amino acid racemization is the chemical reaction which depends on many factors mentioned below.

TEMPERATURE AND PH

Temperature has numerous effects to the ratio of D/L amino acids because racemization is chemical reaction. According to the previous study, they investigated racemization of amino acid in dental enamel. The result found that molars were slightly higher temperature than other teeth because these teeth were located on the back regions in the mouth. Racemization of molars teeth was more rapid than incisors which cooler temperature (Ohtani et al. 2005). This shows that the higher the temperature causes fast racemization rate. Therefore, the specimens should be preserved at low temperatures (below atmospheric temperature) like cadavers preserve at lower temperatures before using for analysis (McCudden & Kraus 2006; Ohtani & Yamamoto 2005).

For pH factor is supported by the previous report; the authors studied aspartic acid racemization in human dentin. They stored the teeth in different solution include acidic, alkaline, distilled water, and in dry conditions. The results showed that the rate of racemization was highest in pH 9 solutions and followed by in distilled water, in pH 4 solution, and in dry condition, respectively. Furthermore, the estimated age from teeth in these environment for 1 year demonstrated that there were slightly increased by about 0.007 years in a dry condition. On the other hand, the teeth left in the pH 9 conditions showed increase of 0.6 years. It is indicated that in alkaline condition is more effective compare to other conditions (Ohtani 1995a).

HUMIDITY

In wet condition, rate of racemization is higher than dry condition. So, the cadavers left in high humidity condition like a tropical rain forest may be not suitable for racemization analysis (Ohtani et al. 2005; Ohtani & Yamamoto 2005).

FIXATION

In another study applied heating experiment was done to determine racemization in teeth of human cadavers. They stored the teeth in different fixatives which were 95% ethanol, 10% formalin solution and 10% neutral formalin fixative at several temperatures. The rate of aspartic acid racemization was highest in 10% neutral formalin solution, followed by in 10% formalin solution, and in 95% ethanol, respectively. Nevertheless, the teeth stored at 15˚C showed that these fixatives almost were unaffected to the amino acid racemization rates (Ohtani et al. 1997).

TYPE OF FRACTIONAL EXTRACTION AND SIZE OF SAMPLE POWDER

There are few studies on amino acid racemization by analysis the fractions of protein. They divided total

protein fraction into two fractions of dentin include acid insoluble fraction (collagen) and acid soluble fractions (non-collagenous protein). The acid insoluble fraction consisted of collagen (Type I collagen fibers). The acid soluble fraction comprised non-collagenous proteins for example osteocalcin, osteopontin, and sialprotein and determined the correlation between the degree of racemization of each fraction and age at death. The results demonstrated that the correlation between the amino acid racemization of the central incisor and age was highest in acid-soluble peptide, followed by in total amino acids, and in acid-insoluble collagen, respectively (Ohtani & Yamamoto 1991). Moreover, there was study aspartic acid racemization in human femur. The results found the D/L ratio of acid-soluble fraction demonstrated the highest correlation with age in males and the racemization rate also was highest in the acid-soluble peptides. It may be caused by collagen is triple helix structure so, it has slower racemization rate (Takagi & Veis 1984). In addition, the different sizes of bone powder particles can be affected to different D/L ratios and found lower D/L ratio of larger particle sizes (Ohtani et al. 1998).

PROTEIN STRUCTURE

Rate of amino acid racemization also depend upon the position of amino acids in sequence of amino acid within polypeptides (McCudden & Kraus 2006) and depend upon the protein structures (Ohtani & Yamamoto, 2005).

Additionally, contamination is one of factors that also affected to D/L ratio of amino acids such as contamination of surrounding connective tissues, blood, and bacterial cell wall. Contamination from other connective tissues and blood increased the amount of L-amino acids (Pfeiffer et al. 1995). In contrast, the cell wall of bacteria contains high the concentration of D-amino acids. So, contamination of bacteria would be increased D-amino acids in sample (Rogers 1983; Schleifer & Kandler 1972). Furthermore, there are other factors (Rajkumari et al. 2013) for example geography, ancestry, lifestyles of dietary and also difference in sex. There were studies on racemization in alveolar bone and it was found that the racemization rate was lower in females because of higher metabolic protein turnover rate (Ohtani et al. 2007).

APPLICATION OF AMINO ACID RACEMIZATION

Amino acid racemization can be used for determine protein ageing. The relationship between amino acid racemization and protein age relies on protein turnover. In high turnover rate of proteins do not accumulate of D-amino acids with age while proteins with low turnover rate show accumulate of D-amino acids with age. In proteins without turnover activity found close correlation between racemization and age (Ritz-Timme & Collins 2002). Moreover, amino acid racemization can be used as biomarker for pathological disorders caused by protein degradation. It occurs due to from abnormal regulation of protein

turnover rate and amount of D-amino acids in tissues change in many diseases (McCudden & Kraus 2006) such as arthrosclerosis, and lung emphysema (Powell et al. 1992; Shapiro et al. 1991).

Racemization of amino acids also applies for age estimation. Bada and Protsch (1973) investigated racemization of amino acids for age estimation in fossil bones. They used aspartic acid racemization to estimate fossil bones age because there were preliminary results of amino acid racemization showed that aspartic acid was fastest racemization rate than other amino acids. This study reported racemization of amino acid can be applied for dating fossil bones and needed to develop method to apply in human (Bada & Protsch 1973).

Helfman and Bada (Helfman & Bada 1975) first applied racemization in living humans. They studied aspartic acid racemization in dental enamel and analyzed protein extracted from 19 samples. The results showed positive correlation between D-aspartic acid concentration and age (r = 0.921) and the D/L ratio of aspartic acid increased with age. These indicates that it can be used the ratio of D/L to estimated age in human. Then, the previous study examined aspartic acid racemization in human dentin and found that the correlation between age and D/L ratio of aspartic acid in dentin is more than enamel (Helfman & Bada 1976). In addition, they also analyzed the concentration of D-aspartic acid in human hemoglobin and found that D-aspartic acids did not increase with age because hemoglobin has rapid protein turnover rate. These studies indicated that it might be possible to utilize aspartic acid racemization in tissues with low protein turnover rate. Then, other studies used this method to estimate age from dentine, enamel, and cementum. The results showed the correlation was similar to the original study (r = 0.991-0.993) (Ogino et al. 1985; Ohtani et al. 1995). This method was more accuracy and the error of estimated age was < 3 years in human teeth (Ogino et al. 1985; Ohtani 1995b; Ohtani & Yamamoto 2010). Aspartic acid racemization from teeth is widely used in age estimation. In the various tissues, teeth are considered as a precise indicator in aspartic acid racemization for age estimation because the teeth are not only most durable after death but also are the organs with low metabolic rates (Arany & Ohtani 2010; Kiran Kumar 2008). Moreover, racemization of aspartic acid has been studied in other parts of human tissues for age estimation in forensic science like bones. The organic matrix of bone composes of about 90% collagen protein and 10% noncollagenous protein like the component in dentin (Ritz et al. 1994; Schulz & Jundt 1989). Bone has more metabolic turnover rate of protein than teeth because it growth, modeling and remodeling. Furthermore, the bone is affected by diseases (Clarke 2008; Ritz et al. 1996).

Ritz et al. (1994) studied aspartic acid racemization in non-collagenous protein of human bone. They used 35 skulls to racemization analysis and found very close correlation between extent of aspartic acid racemization and age in osteocalcin protein (r =

0.99). The conclusions of this study are the following: First, aging process in human organism relates to permanent proteins. These proteins are formed in early life and not exchanged like osteocalcin in bone matrix. Second, racemization can occur in various human tissues such as dentine, bone, and elastin protein in lung. Third, aspartic acid racemization in both bone and permanent protein can be an estimator for protein age and organism age, as well. Thus, the relationship between extent of aspartic acid racemization from bone can be used one of indicator for age determination in forensic context (Ritz et al. 1994). The previous study examined aspartic acid racemization in human bone both sexes. They used 24 cortical part of frontal bone and divided in to acid soluble and acid insoluble fractions for analysis. The results showed rate of racemization in bone less than dentin and acid soluble was highest rate of racemization. It was indicated that protein turnover rate was faster in bone. They believed that acid insoluble fraction (collagen) in bone may be involved remodeling process. Furthermore, higher remodeling in bone may be caused by more physical load from weight bearing of bone. Contamination factor for example connective tissues, blood vessels or bacteria may also effect to D/L ratio of amino acids (Pfeiffer et al. 1995) because the bacterial wall composes of many D-amino acids. (Schleifer & Kandler 1972; Voet & Voet 2004; Zhang & Sun 2014).

In addition, there was preliminary study about racemization of aspartic acid from several types of bone and rib cartilage. They used 10 male cadavers aged between 22-77 years. Samples were obtained from six types of bone and cartilage include the squamous part of temporal bone, manubrium of sternum, spinous process of lumbar spine, lateral part of iliac fossa, sacral spine, femur, and rib cartilage. They examined racemization in total amino acids. They found the correlation between age and ratio of D/L aspartic acid was different. The correlation was highest in sternum (0.974), followed by skull (0.977), and femur (0.985). In contrast to, in the sacral spine (0.739), rib cartilage (0.763), and pelvic bone (0.881) were lower correlation. These results indicated that different kinds of bones are different ratio of D/L amino acids. In this study, they selected only males because females had less D/L ratio of amino acid than males (Ohtani et al. 2002). It may cause by prevalence of osteoporosis and bone disease is more occur in females. Females with menopause and osteoporosis have increasing of metabolic rate (Diaz et al. 1997; Seeman et al. 1989). Remodeling of bone increases in menopause women and continues that has faster rate than women without menopause (Clarke 2008). This result was similar to another study which investigated aspartic acid racemization in human femur. They reported that there was difference racemization rate between genders which was better in males (Ohtani et al. 1998).

There are other structures which used in age estimation by aspartic acid racemization e.g. articular cartilage, intervertebral disc, yellow ligament,

Table 1: Summary of coefficient of correlation between D/L ratios and ages in various human structures for age estimation from aspartic acid racemization

Author	Structure	Sample no.	Coefficient of Correlation
Helfman and Bada (1975)	Enamel	19	0.921
Helfman and Bada (1976)	Dentin	20	0.979
Ritz & Schutz (1993)	Intervertebral disc	68	0.970
Ritz et al. (1994)	Skull	35	0.990
Ohtani et al. (2002)	Sternum	10	0.974
	Skull	10	0.977
	Femur	10	0.985
	Sacral bone	10	0.739
	Pelvic bone	10	0.881
	Rib cartilage	10	0.763
Verzijl et al. (2000)	Articular cartilage	23	0.950
Ritz-Timme et al. (2003a)	Yellow ligament	46	0.84-0.92
Powell et al (1992)	Aorta	12	0.900
Ritz-Timme et al. (2003b)	Skin	-	0.980
Shapiro et al. (1991)	Lung parenchyma	14	0.980
Masters et al. (1978)	Eye-lens	17	0.912

skin, lung parenchyma, aorta, and eye-lens. They found correlate of racemization and age. The correlation coefficient of D/L ratio of aspartic acid and age in these tissues varies between 0.70-0.99 (Maroudas et al. 1992; Masters et al. 1978; Powell et al. 1992; Ritz-Timme et al. 2003a; Ritz-Timme et al. 2003b; Ritz & Schütz 1993; Shapiro et al. 1991; Verzijl et al. 2000). Summary of correlation between age and aspartic acid racemization for age estimation in different human organs was tabulated (Table 1).

AMINO ACID RACEMIZATION METHODS

In this review, we focussed on the aspartic acid racemization in both teeth and bones. The procedure of amino acid analysis compose of sample preparation, hydrolysis, derivatization, and quantification of amino acids.

SAMPLE PREPARATION

There were studies in many parts of teeth such as enamel, dentin, and cementum. Among these parts of teeth, dentin is usually used for age estimation because of most accuracy, durable and less contaminate to environment or oral cavity. Besides, dentin was formed in early life and low metabolic turnover rate (Arany & Ohtani 2010; Griffin et al. 2008a; Robins et al. 2001). Most preparation method of teeth used sectioning or pulverization of sample for preparation step. The previous study applied aspartic acid racemization in 61 teeth from cadavers (Ogino et al. 1985). This method was briefly as follows:

1) cleaned soft tissues; 2) separate crown and root of teeth by using discs and burs cooled; 3) separated dentin from enamel; 4) coronal dentin was cleaned in distilled water and 0.2M HCL; 5) rinsed with distill water 3 times; 6) used the mortar and pestle to powdered sample; 7) powdered dentin was prepared to hydrolysis step.

Another study investigated (Yekkala et al. 2006) racemization of aspartic acid from human dentin for age estimation. They collected premolar teeth for analysis. This method was briefly as follows: 1) used a slow-speed diamond saw to cut longitudinal section of 1 mm thickness; 2) removed cementum and enamel; 3) dentin samples were powdered by a vibratory mill; 4) sample were demineralised in Na_2EDTA with NaN_3; 5) shaken sample for 2 hr and centrifuged; 6) collect the sediment and rinsed with water; 7) centrifuged again to remove residues of EDTA; 8) sediment (dentin collagen) sample was prepared to hydrolysis. For aspartic acid racemization in bone, the preparation step was similar to the teeth. Ohtani et al. (2002) studied age estimation by using racemization in total amino acid of many kinds of bone. The preparation method was briefly as follows: 1) cut bone with anatomical saw; 2) divided bones into pieces about 1 cm^3 by a low-speed saw; 3) bone surface was polished by a whetstone to remove soft tissue; 4) clean sample in distilled water ethanol and ether with ultrasonic washer; 5) left sample to dried; 6) powdered the bones by using grinder; 7) powdered sample was prepared to hydrolysis.

Then, the previous study determined extent of racemization in aspartic acid, glutamic acid and alanine from human femur (Ohtani et al. 2004). They investigated three part of protein fraction of each amino acid include total amino acid fraction (TAA), acid insoluble collagen fraction, and acid soluble peptide fraction. The preparation method was briefly as follows: 1) cut the middle part of femur with cutter; 2) bone surface was polished by a whetstone to remove soft tissues; 3) removed spongy bone to obtain only compact bone; 4) cut bone into pieces about 4 mm^2; 5) cleaned bone sample in distilled water, ethanol and ether with ultrasonic bath for 5 min; 6) left sample to dried; 7) powdered the bones by using mortar; 8) powdered sample was divided to IC and SP fractions using 6 M hydrochloric acid; 9) centrifuged for 1 hr and left to dried; 10) all sample were prepared for further hydrolysis.

Most preparation methods of aspartic acid racemization used grinding or section of the teeth or bones for extracting protein fraction. However, there is a new approach for amino acid racemization which is less destructive method. This method was used acid dissolution to extract proteins from the dental enamel for minimize the amount of destruction of the teeth (Griffin et al. 2008a; Griffin et al. 2008b). They studied racemization in dental enamel. The result has found good correlation between D/L ratio of aspartic acid and age at death. This method only extracted the acid soluble fraction of enamel which forms in early life and also has low metabolic turnover of protein. Most

of noncollagenous proteins (acid-soluble fraction) can be extracted from enamel by using acid etching (Fisher & Termine 1985; Schulz & Jundt 1989). The preparation method was briefly as follows: 1) removed all connective tissues attachment and cleaned by 6 M hydrochloric acid filled with 0.2 ml PCR tube for 1 min; 2) rinsed by HPLC grade methanol; 3) placed sample in 12% sodium hypochlorite for 2 days; 4) rinsed in ultrapure water and HPLC grade methanol again; 5) added 6 M hydrochloric acid filled with 0.2 ml PCR tube for two consecutive time interval of 1 min; 6) obtained protein fraction; 7) prepared to further amino acid analysis. This approach preserved whole teeth so teeth can be used for further analysis. Using acid dissolution to removed contamination of tooth surface and obtained extracting protein fraction for analysis (Griffin et al. 2008b).

ANALYSIS METHOD FOR QUANTIFYING D-AND L-AMINO ACIDS

Most of analysis methods for separation of D-and L-amino acids have used chromatography approaches which are gas liquid chromatography (GC) and high pressure liquid chromatography (HPLC). All chromatographic techniques consisted of three contents: the stationary phase, the mobile phase, and the detector. Chromatography is used separation sample mixture which occurs between the mobile and stationary phases. GC method uses gas for the mobile phase such as helium or nitrogen (Robins et al. 2001). HPLC method is divided into normal phase and reverse phase liquid chromatography. For normal phase or ion-exchanges system (Hare et al. 1985), the mobile phase is non-polar solvent and the stationary phase uses polar with polysulfonated cation-exchange resin. In contrast to reverse phase liquid chromatography, there are using non-polar in the stationary phase and a polar solvent for the mobile phase (Johnson & Miller 1997). Both methods have general used for separation amino acids but gas chromatography has expensive cost and complicate to preparation of sample (Qudsia et al. 2014). HPLC method is usually applied in racemization method in recent times (Fu et al. 1995; Yekkala et al. 2006). There are several advantages of HPLC method such as high sensitivity, shorter analysis time, simple procedure, high reproducibility, lower cost (Bene ová et al. 2004; Ogino & Ogino 1988; Qudsia et al. 2014; Yekkala et al. 2006). The data obtained from detector system was the chromatogram.

CONCLUSION

Aspartic acid racemization is natural process which takes place in stable protein of organism. Interconversion of amino acids occurs slowly at the body temperature and depends on temperature, pH, humidity etc. Increasing amount of D- aspartic acids is useful estimator for aging of individuals. Racemization of aspartic acid can be detected in many tissues such as teeth, artery, cartilage, and bone. For teeth and bone, there are different preparation methods of aspartic acid

racemization which include grinding, section or acid etching of specimen to extracting protein fraction. These methods provide varied correlation and accuracy for age estimation.

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Received: 17 December 2016
Accepted: 6 July 2017