Sensing of Essential Amino Acids Behaviour Under Fast Thermal Shocks in Liquid Water Environment

Michal Borecki, Jan Szmidt
Institute of Microelectronics and Optoelectronics
Warsaw University of Technology
Warsaw, Poland
borecki@imio.pw.edu.pl

Mariusz Duk, Andrzej Kociubiński
Lublin University of Technology
Department of Electronics
Lublin, Poland
akociub@semiconductor.pl

Michael L. Korwin-Pawlowski
Département d'informatique et d'ingénierie
Université du Québec en Outaouais
Gatineau, Québec, Canada
Michael.Korwin-Pawlowski@uqo.ca

Tomasz Niemiec, Maciej Szmidt, Kaja Urbańska
Warsaw University of Life Sciences
Warsaw, Poland
t_niemiec@tlen.pl

Przemysław Prus, Elżbieta Prus
Forcate
Wroclaw, Poland
przemyslaw.prus@forcate.pl

***Abstract* - Essential amino acids exist normally in solid state, but in the environment they are often in contact with water or other liquids. The stability of solid state amino acids has been investigated under controlled atmosphere and pressure conditions. The amino acids stability in liquid water environment is important, especially for protein permanency and for human and animal food usage. In this work, the full set of essential amino acids are investigated in water environment at normal atmospheric pressure and under thermal shocks with slew rate as high as 4ºC/sec applied in a fiber optic capillary sensor set up. Reactions in some essential amino acids happen at temperatures lower than 80°C. The conclusion is that the stability of amino acids in water environment is different from their stability in solid state form.**

Keywords-essential amino acids; amino acids in water; L-lysine; capillary sensor

I. INTRODUCTION

A. Essential amino acids characteristics

Amino acids contain amine (NH_2) and carboxylic acid (COOH) functional groups, along with a side-chain specific to each amino acid, as shown in Figure 1. Amino acids are the basic bioelements of proteins, important macromolecules for the functions of humans and animals.

Amino acids are built of structural units called monomers that can join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. Amino acids that are precursors to proteins are called proteinogenic amino acids. There are 23 of proteinogenic amino acids, while 20 of them are directly encoded by the universal genetic code. Humans can synthesize 11 of these 20. The other 9 are called essential amino acids and must be consumed in the diet. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The essential amino acids in pure form exist in solid state. In the gas environment, the interactions of amino acids are enabled by Van der Waals forces, by electrostatic charge forces and, in some cases, by the single sigma bond that exists between the hydrogens. In the water environment, amino acids interactions are influenced by the electrostatic charge of the water particles and by the hydrogen bonds. The general properties of amino acids are presented in Table I.

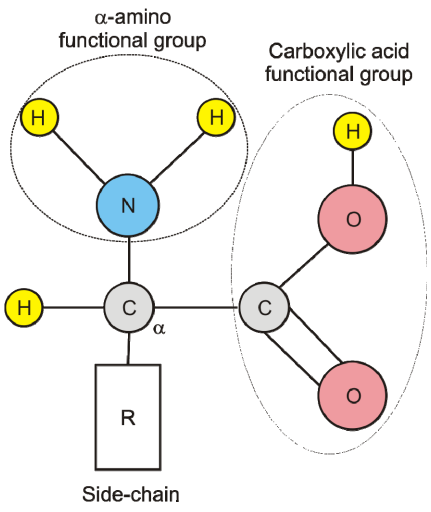

Figure 1. Amino acids structure.

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TABLE I. GENERAL PROPERTIES OF AMINO ACID

Amino-acid	Molar mass [g mol^{-1}]	Approximate melting point [°C]	Solubility in H_2O at 25°C [g/100 mL]
leucine	131.17	286	2.43
lysine	146.19	220	150
valine	117.15	298	8.85
phenylalanine	165.2	275	2.96
isoleucine	131.17	288	4.12
threonine	119.12	270	9.0
methionine	149.21	258	3.38
histidine	155.16	284	4.19
tryptophan	204.24	282	1.14

Amino acids are usually classified into groups according to the properties of their side-chains. Depending on the side-chain, an amino acid dissolved in liquid water environment could form a weakly acidic or a weakly basic solution. An amino acid is a hydrophilic substance when its side-chain is polar or carries a charge in liquid water environment. It would be a hydrophobic substance if the side-chain was nonpolar or neutral [1]. The effect of liquid water on the structure of amino acids is important because liquid water takes part in a wide range of reactions within a biological cell, and is an integral part of all proteins.

The amino acids side chains have a strong influence on how the protein behaves in liquid. For example, the structure of the lysine molecule in liquid differs for different charge states [2]. Below pH 9 both amino groups are protonated. Around pH 9, the α-amino group is protonated and the amino group of the side chain is not. Above pH 10.5 both amino groups are deprotonated. Therefore, lysine in drinking water is characterized by both amino groups protonated.

The requirements of essential amino acids of humans and the efficiency of amino acids in protein utilization at maintenance and sub-maintenance levels have been investigated [3]. Amino acids in the form of proteins are the second-largest component (water being the largest) of human muscles, cells and other tissues. Along with proteins, amino acids perform critical roles in the processes of neurotransmitter transport and of biosynthesis. The essential amino acids requirements in people's diet change significantly with their age [4].

The indicative values of amino acids diet requirement and amino acids composition of body protein are presented in Table II. Because it has to be present in food, lysine is one of the most interesting of essential amino acids. In many cases, lysine is supplemented into the animal and vegetarian feedstuffs [5].

Purified lysine is safe for human. Conventional dosages of lysine supplementation are of 3 to 6 grams daily [6]. One common use for lysine is the treatment of cold sores caused by herpes virus [7].

TABLE II. DIET REQUIREMENTS FOR ESSENTIAL AMINO ACIDS OF HUMANS

Essential amino acid	Requirement (mg/kg per day)	Amino acid composition of body protein (mg/g)
leucine	39	98
lysine	30	78
valine	26	56
phenylalanine	25	53
isoleucine	20	46
threonine	15	34
methionine	15	22
histydyne	10	32
tryptophan	4	0 (precursor of serotonin)

B. Essential amino acids examination in water enviroment

The thermal decomposition of amino acids depends on the environment [8]. The study of the thermal decomposition of amino acids in water environment is important, because it would allow refining the protein degradation model [9]. So far, the decomposition behavior of amino acids has been selectively investigated in water at high-temperature and high-pressure in a continuous-flow tubular reactor. The reactions were carried out in the temperature range of 200-340°C at a pressure of 20 MPa [10]. The mechanisms of heat damage in proteins when amino acids were in contact with water have been also examined. It was shown that lysine's disintegration may start when the temperature exceeded 37°C [11].

In this work, the possibilities of thermal decomposition of essential amino acids in water environment in conditions simulating those typical for some methods of thermal preparation of food were examined.

The paper consists of 5 sections. First section was the introduction where the amino acids properties were discussed and the aim of work is presented. Second section describes the proposed for this purpose experiment set-up. The examination results of essential amino acids are presented in section three. In the section four, the discussion of obtained results and their classification is proposed. The short conclusion of proposed examination is gathered in section five.

II. EXPERIMENT SET-UP

To perform our experiments we used a photonic capillary sensor capable of producing a temperature ramp of over 4°C/sec in a liquid water environment. The sensor head schematic side cross-section is shown schematically in Figure 2. The light traces, from source to receiving fibers, for bubble presence, are shown schematically in Figure 3. In this situation, the received signals are minimal. When capillary is uniformly filled with liquid for the light travelling from source fiber the light refraction in liquid occurs, and reflected ray reaches received fiber.

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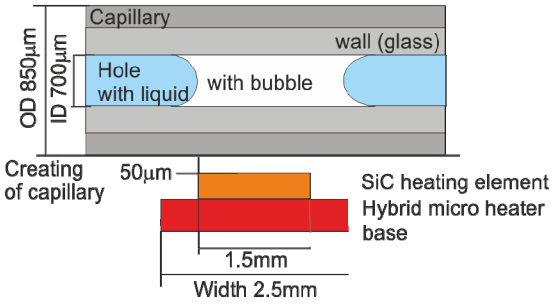

Figure 2. Schematic side cross-section of the capillary head.

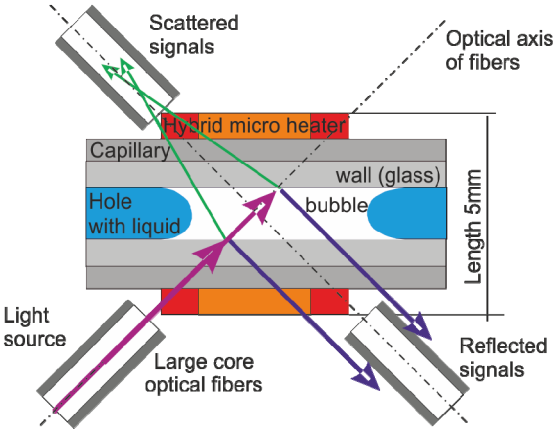

Figure 3. Schematic top view of the capillary head.

Therefore, the sensor works on the principle of optical intensity changes taking place in dynamically forced measurement cycles. The sensor monitored simultaneously the thermodynamic and optical parameters of liquids [12]. It used an optical capillary in which the phase change from liquid to gas (a creation of a gas bubble in the liquid) was forced by local heating while the propagation of light across the capillary was monitored. After the bubble of gas was created, the local heating was switched off, but the bubble expansion still forced the flow of the liquid. The forced liquid flow in the capillary depended strongly on the vapor pressure and could be classified by a cavitating or non-cavitating type [13]. After the local heating, the gas phase could be absorbed in the liquid, or remain in the form of a small bubble. As a result of the decomposition of the components of the liquid, or alternatively, when the forced flown was turbulent or of the cavitating type, a series of air bubbles might exist in the liquid.

The capillary head used in this work was a modified version of one presented by Borecki et al [14]. The detection of the reflected signal was supplemented with the detection of scattered signal. The optical fibers were moved to the position in which their axes crossed in the central intersection point of the hybrid micro heater and the capillary. That enabled the monitoring of precipitation of the solution's components. Instead of the 2.5mm wide element using for the micro-heater [14], a new 1.5mm wide SiC heating element was developed. This way, the local heating area dimensions get closer to the dimensions of the optical beams than in our previous investigations. The TSP 700-850 from Polymicro Inc. capillaries were cut into 10 cm sections. In experiments capillaries with both ends closed were used. On the measuring head large core optical fibers BFH 37-600 from Thorlabs were installed.

As light source, a fiber coupled laser device S1FC675 from Thorlabs was applied. The diode wavelength was 675nm. The source was electrically modulated with 1kHz frequency from a Rigol function generator DG2021. The optical power at the end of light source fiber was set to 0.1mW, by using the modulation signal amplitude, offset and knob of laser device.

The optoelectronic detection unit coupled to large core optical fibers was of our own developed construction. It is modified version presented in [14]. It is characterized by switchable from 10nW to 1mW detection optical range. Lower detection threshold, for output refresh rate less than 100Hz, is 50pW. The response time, for full scale signal change, is lower than 10ms. The optoelectronic unit was connected to a PC by an analog input of IOtech Personal daq 3000 data acquisition system. That system was also used to control the laboratory power supply HM8143 of the micro heater. The micro heater was powered with 5W in periods shorter than 30s. To operate the system, at a 0.1s sampling rate, script in DasyLab 10 was designed.

The initial experiments were made using capillaries filed with deionized water, as presented in Figures 4 and 5. As can be seen in Figure 4, the scattered signal was constant during the sample's local heating, meaning that precipitated components were not present in the water. The presence of a moving boundary between the gas and the liquid phases can be correlated with the peak in scattered signal. The deionized water boiled in the measurement cycle in the time range from 17.1 to 25 seconds, corresponding to a boiling temperature that could be explained by the superheating of water, when at atmospheric pressure it could reach 120°C. The conditions for superheating of water are fast heating of a not vibrating sample of clear water in a vessel with smooth walls. Those conditions were maintained in the analyzed measurement cycle.

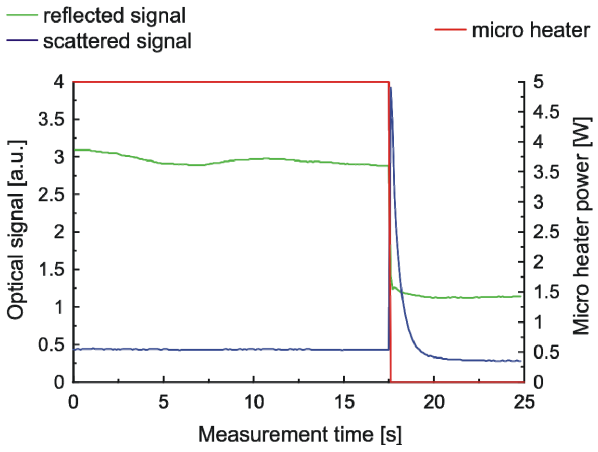

Figure 4. Measurement cycle of deionized water showing the reflected and the scattered signal during heating.

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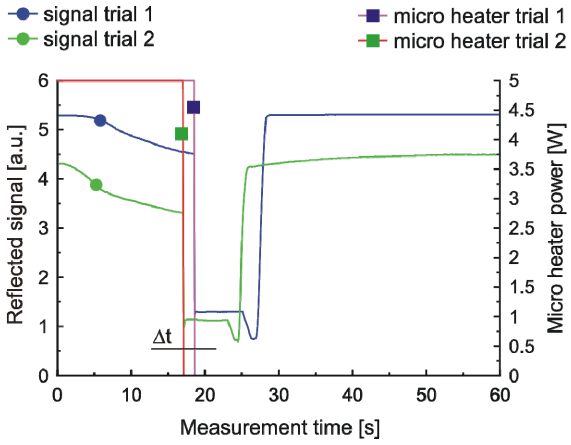

Figure 5. Measurement cycle of deionized water, two trials with characteristics repeatable shape of signal.

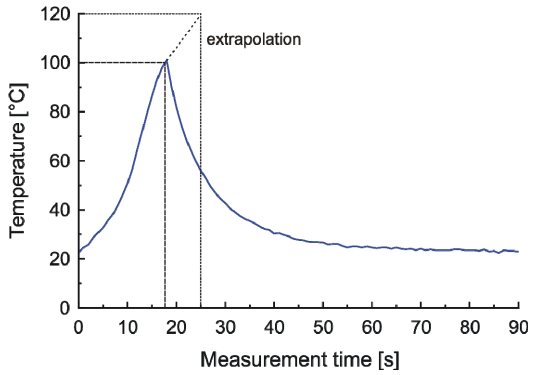

Figure 6. The maximum local temperature of deionized water in the capillary during a measurement cycle.

During the calibration of the sensor the temperature distribution in the capillary optrode was registered with the use of R300 NEC thermo-vision camera and the InfReC software. The measured and calculated water temperatures during the measurement cycles are presented in Figure 6. The obtained results confirm boiling at 100°C in 17.1 seconds of local heating. The extrapolation of the results showed that the temperature of superheating of water in the analyzed conditions did not exceed 120°C.

III. EXPERIMENTAL RESULTS

Deionized water was used for making solutions at 100mM/dm^3 concentrations containing amino acids of 99% Reagent Plus purity obtained from Sigma Inc [15]. Ten experiments series for each examined amino acid were performed. Scattered signals during local heating in all experiments were stable so solid state precipitates did not form. Therefore, these signals are not presented in following records. The random selected two characteristics of experiment series refracted and reflected signal, for each examined amino acid, are presented in subsequent figures.

The amino acid simplest in structure is glycine. It is not an essential amino acid. Its solubility in water is 24.99g/100 mL at 25°C and the decomposition temperature of glycine was about 230°C.

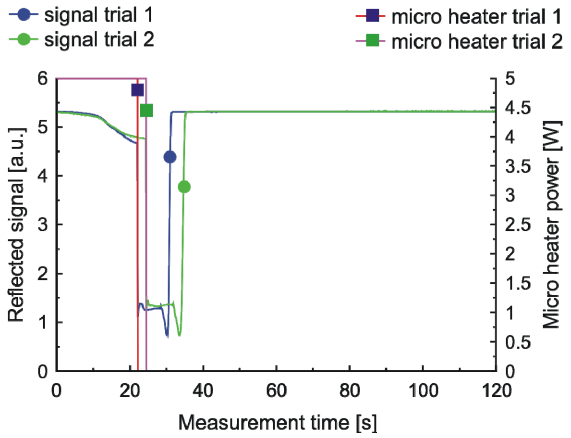

Figure 7. The measurement cycle of glycine

A solution of 100 mM/dm3 of glycine in water was used for the measurement cycles presented in Figure 7. The glicine measurement cycle was similar to that of water, as was expected, because the solution of glycine in water was uniform, its concentration did not approach the solubility limits.

A. *Examination of essential amino acids*

The measurement cycle of histidine is presented in Figure 8. The histidine solution starts boiling in 15.8-17.1 seconds of the measurement cycle, slightly before sample reached 100°C. In our opinion, the gas product of decomposition had a relatively higher pressure than that of water steam. This gas phase caused turbulent flow of liquid, as could be concluded from the highly non monotonic measurement cycle characteristic observed after the micro heater switch off.

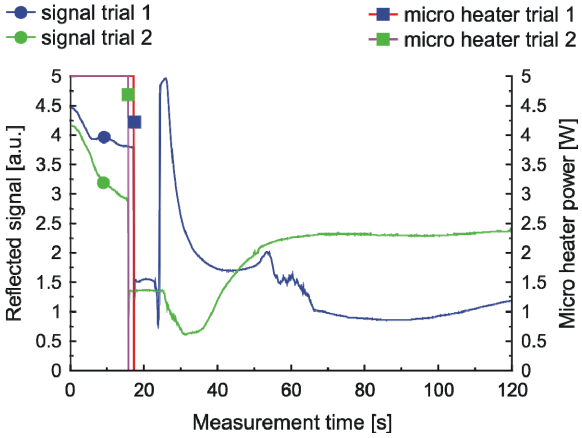

Figure 8. The measurement cycle of histidine

Isoleucine ($C_6H_{13}NO_2$) in water solution generated the gas phase in the range of 6.3-11.3 seconds of the measurement cycle, which was faster than water and histidine. The gas phase was next absorbed, as depicted in Figure 9. Leucine ($C_6H_{13}NO_2$) measurement cycle is shown in Figure 10.

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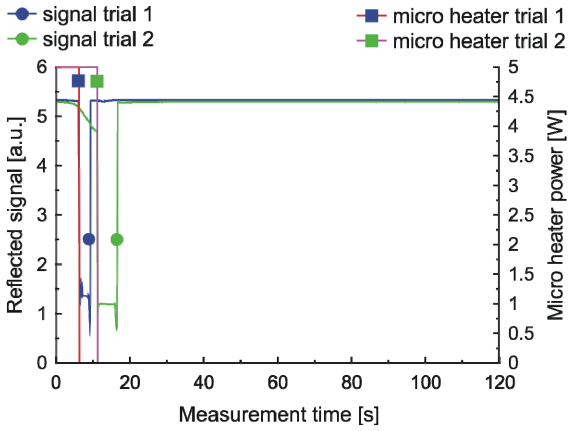

Figure 9. The measurement cycle of isoleucine

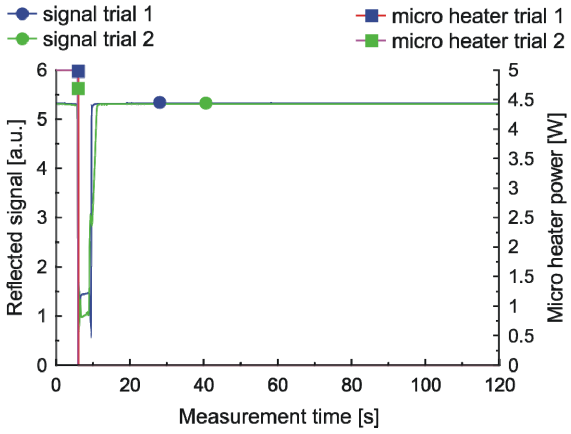

Figure 10. The measurement cycle of leucine

The measurement cycles of leucine had the same course as isoleucine, with the gas phase appearance in 6 seconds. The measurement cycles of isoleucine and leucine were similar, probably due to the small difference of their structures as their molecular formula is the same.

The measurement cycle of lysine is presented in Figure 11.

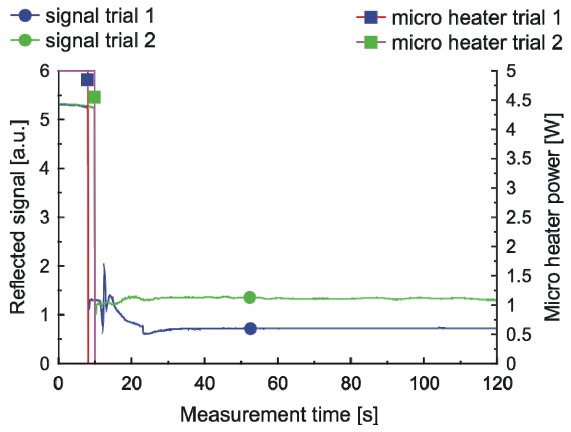

Figure 11. The measurement cycle of lysine

Lysine ($C_6H_{14}N_2O_2$) has the same carbon number as isoleucine and leucine, but measured characteristics differs significantly. The generation of the gas phase in the lysine solution was the most violent of the examined essential amino acids. It caused the cork of the capillary to shoot out in each measurement cycle. Therefore, the stable low level signal after the shut off of micro heater can be observed. The gas phase generation occurred in the range from 8.1 to 9.0 seconds of the cycle.

The methionine measurement cycle of Figure 12 shows similarity with the water cycle of Figure 5.

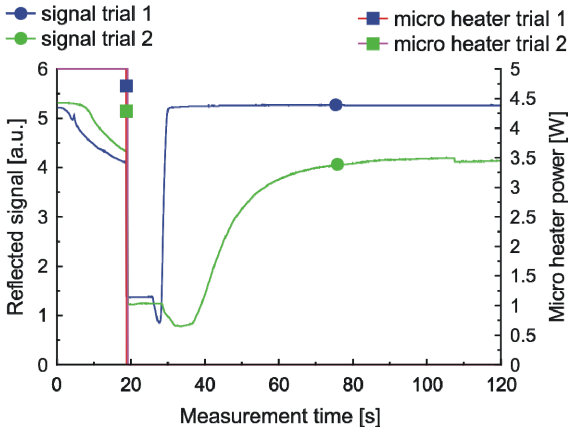

Figure 12. The measurement cycle of methionine

Phenylalanine was characterized in the measurement cycle by a rapid gas phase creation in 9.4-9.8 seconds, as shown in Figure 13.

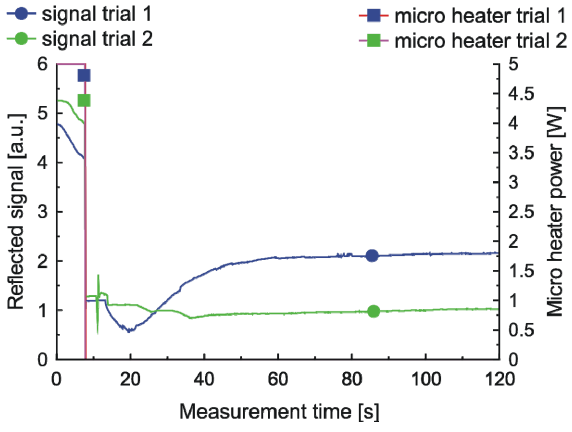

Figure 13. The measurement cycle of phenylalanine

Threonine decomposition occurred in 15.5-16.3 seconds of the measurement cycle, with a turbid flow of liquid, as presented in Figure 14.

The measurement cycle of tryptophan of Figure 15 is similar to the cycle of water.

Valine has the measurement cycle similar to water and tryptophan, as depicted in Figure 16.

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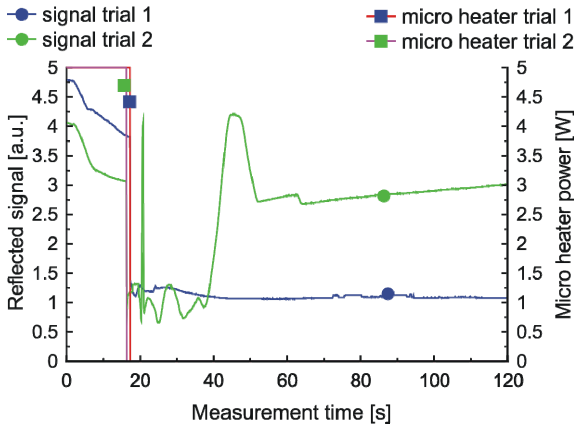

Figure 14. Measurement cycle of threonine

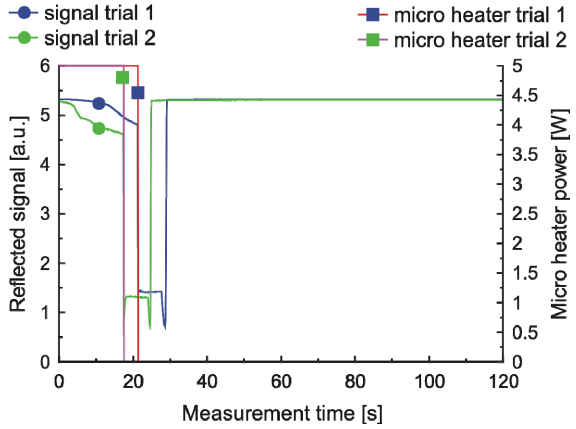

Figure 15. The measurement cycle of tryptophan

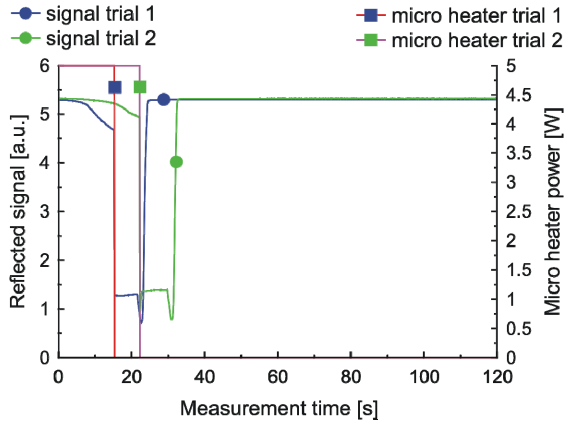

Figure 16. The measurement cycle of valine

IV. DISCUSSION OF RESULTS

Sensing of essential amino acids under fast thermal shocks in water environment shows that the amino acids solutions behavior can be described according to the form of gas phase creation as 1) similar to water (H_2O-like); 2) early; and 3) impetuous and early, as listed in Table III. The water-like behavior can be described as one with the bubble appearance later than 16 seconds of the measurement cycle. The early gas phase creation can be described as when the gas phase creation happens before 16 seconds of local heating - below 80ºC. Impetuous and early gas phase creation occurs when, additionally, the gas phase forces rapid or turbid solution flow.

TABLE III. SUMMARY OF RESULTS OF ESSENTIAL AMINO ACIDS EXAMINATION

Amino acid	**Type of amino acid in water**	**Relation to water**	**Avg. time of rapid thermal reaction [s]**	**Type gas phase creation**
leucine	neutral	hydrophobic	6.0	early
lysine	charged	hydrophilic	8.5	impetuous and early
valine	neutral	hydrophobic	18.8	H_2O like
phenyl-alanine	neutral	hydrophobic	9.6	impetuous and early
isoleucine	neutral	hydrophobic	8.8	early
threonine	polar	hydrophilic	16.0	H_2O like
methionine	neutral	hydrophobic	19.0	H_2O like
histidine	charged	hydrophobic	16.4	H_2O like
tryptophan	neutral	hydrophobic	19.4	H_2O like

Four distinct cases are of interest. The early gas phase creation of leucine and isoleucine happens because they have similar structures and parameters. The impetuous and early gas phase creation happens for lysine and phenylalanine.

On the other hand, the degradation of amino acids in water environment for situations involving enzymatic oxidation or hydrolytic deamination is well-known [16].

V. CONCLUSION

Developed capillary sensor set-up enables sensing of essential amino acids behavior under fast thermal shocks in liquid water environment. Experiment show that, a fast temperature increase may act as a reaction catalyst for the disintegration of some amino acids, particularly lysine. This can be used for explaining, efficiency of herpes virus curing with lysine supplementation in some group of peoples. This can also clarify traditional medicine recommendations of raw parsley and chives consumption, which can be compiled with highest in vegetables lysine concentration [17]. Therefore, food preparation processes with fast ramp ups of temperature, are not ideal when the food is to be a source of amino acids. Consequently, we intend to examine amino acids stability in oil environment as some oriental kitchen methods base on putting shredded food into boiling oil or heated clarified butter which may lead to proven lysine deficit of India people [18].

The phenomena of lysine disintegration may be used in new nanotechnology medical or diagnostic particles carriers using graphene oxide [19]. That effect will be the subject of

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our further studies. We will concentrate on examining the presence of products while heating graphene oxide - amino acids solutions in liquid water environment.

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