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The super molecular structure in eggs biomatrices from free-range and battery hens as investigation platform for long time space flights

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ABSTRACT

85 wt. % of the structured water and 100 wt. % of solvated ion pairs' clusters in egg yolk from free-range hens (FH) are at temperatures lower than 298 K in special depositories where theese depositories shall be destroyed at temperatures >300 K. The small water clusters $(H_2O)_{11\pm1}$ interact differently with the protein bases depending on the temperature of the eggs. The influence of captive breeding (CB) on the eggs biomatrix was shown and compared with that one of FH. CB eggs don't have any maximum of collapsed clusters at 300 K (egg-white) and 305 K (egg yolk). Additionally, the statistical cluster ensembles in CB eggs are stable up to 357 K whereas in FH eggs they depend on temperature. Comparing CB with FH it was found that for the first one the number of the main protein coils after the quarantine was highly reduced. The state of ovalbumin monomers, dimers and octamers and the state of their surroundings in biomatrices was discussed. Information about the water content in ovalbumin monomers and octamers and about the interaction of these protein associates with the surroundings is given too. The difference between the free-range and battery eggs biomatrices could be helpful to later investigations concerning long time space flights.

KEYWORDS

Eggs;
Biomatrix;
Stress;
Ageing;
Ovalbumin;
Structure;
Long time space flights.

INTRODUCTION

The structure of egg-white and egg yolk at the level of cluster ensembles and micro domains isn't cleared up completely hence there isn't any method for a fast and safe determination of statistical ensembles. Using the Zubow gravitation mass spectroscopy (ZGMS) it is possible to investigate the long-range order (LRO) in liquids, in protein coils and protein coil associates in solutions[1, 2, 3]. By the German company "AIST Handels- und Consulting GmbH", this method was suggested to be applied for the LRO analysis of liquids, clusters in proteins as well as for their interaction with the surroundings[4, 5]. Statistical ensembles in liquids, seed crystals, clusters and protein coil associates play an important role in the early stage of the embryo origin.

The aim of the present work is to apply the multidimensional digital signal processing[6, 7] to the investigation of the biomatrices in hens' eggs as well as to find out whether there are differences between the eggs from free-range hens and battery hens (bird flu, quarantine 2005 in Germany).

MATERIALS AND METHOD

To analyze egg yolk and egg-white, fresh eggs from free-range hens (10 pieces, June 2005) and battery hens (10 pieces, 45 days after the beginning of the quarantine, October … December 2005) were chosen. The egg yolk was separated from the egg-white and placed in the measurement cell of the ZGMS of the German firm «Aist Handels- und Consulting" GmbH where the spectra were recorded with a scanning time of 30 s.

Every measured signal was cleaned from gravitation noises raised by proton dissolving/condensation in physical vacuum[8] and analysed. The experiments were repeated three times. The measuring error was 10 ± 5 %, the temperature stability ± 0.1 Ê and the ZGM-sensor was installed directly in the egg yolk (in vivo, Figure 1).

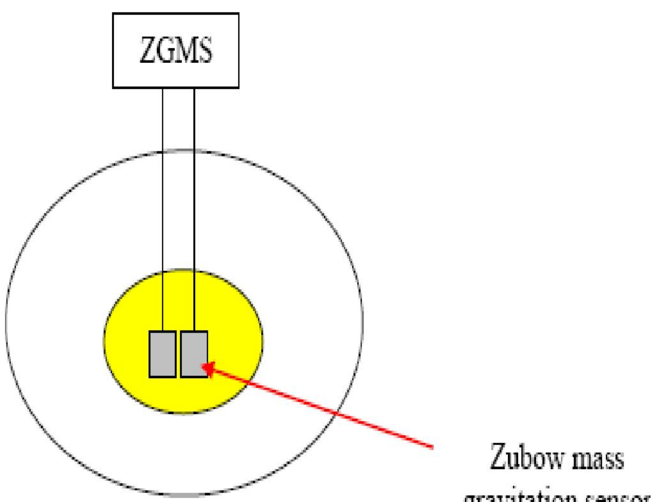


Figure 1 : Measurement scheme.

Device calibration and measurement procedures were carried out as described in[6]. The ZGMS-spectroscopy is based on known methods of the statistical analysis of very weak gravitation signals[1]. Yamauchi[9] suggested a new analogous analysis method for molecule oscillations with intramolecular and/or intermolecular energy transfer that is given in time-frequency characteristics. Using this method, which is based on short-time Fourier transformation, energy density fluctuations can be detected too just like with ZGMS. In the following, it shall be described how real cluster signals were cleaned from noises of the measurement cell[5].

RESULTS AND DISCUSSION

As shown in Figure 2, the main clusters are represented as solvated clusters of ion pairs (SCIP) in egg yolk at 289 K[6]. Since the SCIP signals are considerably stronger than those of the other oscillators they overlap these. The presence of high molecular SCIPs could be an indication for that these clusters are confined in limited spaces. At heating the egg yolk, the SCIP signals disappeared however new signals typically for organic clusters appeared[7]. Probably, cations (metals, ammonium) in SCIP participate in the formation of new oscillators on the base of proteins and they could act as catalyst in the beginning biochemical reactions of the embryo growth. The oscillator signal of the small expanded water cluster $(H_2O)_{11\pm1}$ is observed to be reduced by almost six fold (http://www.lsbu.ac.uk/water/index.htm[10]). Before heating, water clusters and SCIPs are in an intermolecular hydrophilic space of the biomatrix, with heating the surroundings has been changed from hydrophilic molecules to hydrophobic ones. The contact of water clusters with the hydrophobic lipids leads to a weakened interaction with surroundings and the water clusters implode in the collapsed form. Now, with increasing temperature, the structured water and SCIP start to participate actively in biochemical reactions of the embryo growth. Therefore, at low temperatures, the salts of egg yolk are deposited in SCIPs and water appears mainly as small clusters. The nano emulsion in egg yolk seems to be destroyed at temperature increase and its destruction products take part in biochemical processes of the embryo growth.

As shown in the Figures 2 and 3 the ZGMS-spectra of egg-white and egg yolk are strongly different. One sees this particularly at the ZGMS-spectrum of egg yolk at 289 K, which is typical for SCIP[6]. According to Belitz[12] the salt content in egg yolk and egg-white amounts to 0.6 and 1.1 wt. %, respectively. If salts are available as oscillating SCIPs then the SCIP signals could influence the complete spectrum and over-

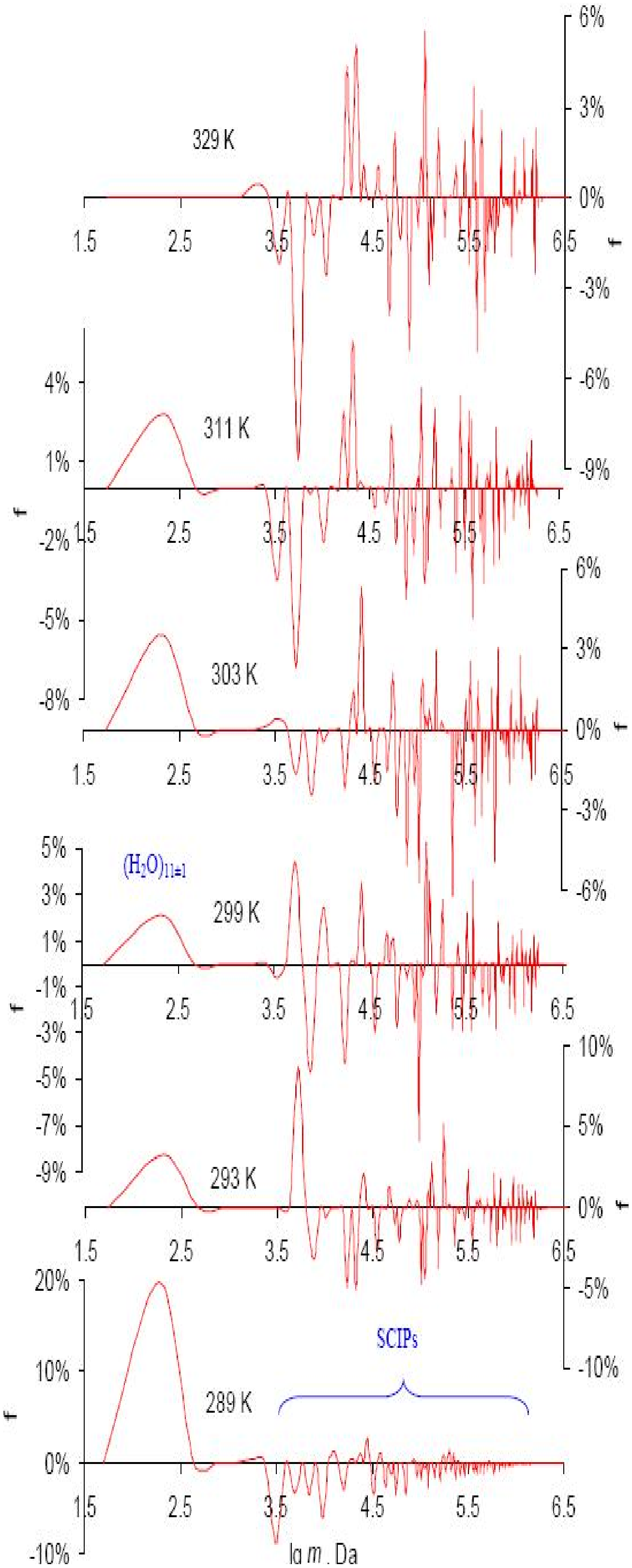


Figure 2 : ZGMS-spectra of egg yolk at different temperatures. Free-range eggs. Strong shock waves. Zubow constant $6.4\cdot10^{-15}$ N/m[11]. The value *f* reflects the energy characteristics of an oscillator.

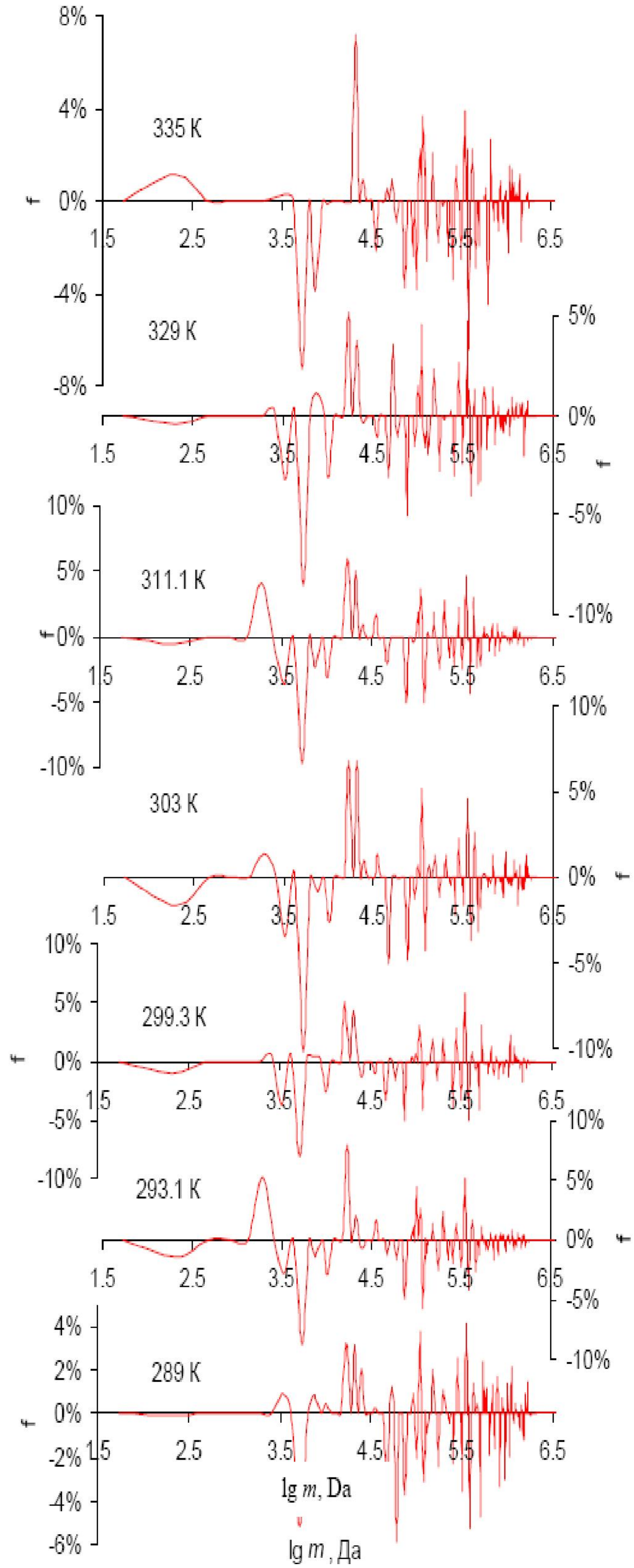
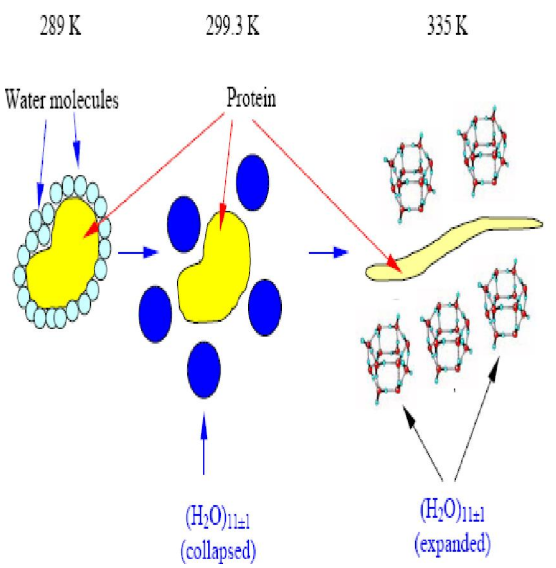


Figure 3 : ZGMS-spectra of egg-white at different temperatures. Free-range eggs. Strong shock waves. Zubow constant $6.4\cdot10^{-15}$ N/m.

lap the other signals. With temperature rise these signals disappeared and consequently the SCIPs. The signal of $(H_2O)_{11\pm1}$ is recorded as expanded oscillator in egg yolk (Figure 2) and it disappeared at 329 K only, explained either by that the small water clusters were included in biochemical processes or by that a new thermodynamic balance with the collapsed clusters was achieved[6]. In contrary, in egg-white, these water clusters were found to be in the collapsed form that was transformed to the expanded one at 335 K (Figure 3). The concentration of the base water cluster $(H_2O)_{11\pm1}$ is very low at 289 K in egg-white; water could be in non-oscillating structures, for example as adsorption water on protein molecules. Water desorption from protein molecules at low heating (299.3 K) leads to the formation of collapsed water clusters near to the proteins (scheme, Figure 3). At 335 K, the hydrogen bonds between the nitrogen bases were destroyed because of a beginning protein denaturation. This process gives new possibilities for new hydrogen bonds with these collapsed water clusters, accompanied with their immediate transition to the expanded state (scheme, the model of the base water cluster is given with kind permission of prof. Lenz A.[13]). Thus, the evolution of the water cluster structure and its important role in biological systems can be studied.



scheme

As we found out earlier, the cluster structure depends on temperature, pressure, mechanical fields[4], chemical/biochemical substances as well as on weak gravitational fields[5]. Furthermore, ensembles of clusters in liquids were observed to influence biological processes[14], particularly under space conditions (space station) and under the origin of the life on other planets, we believe.

The other ZGMS-signals in the Figures 2 and 3 reflect the oscillations of domains, macromolecule coils and coil associates.

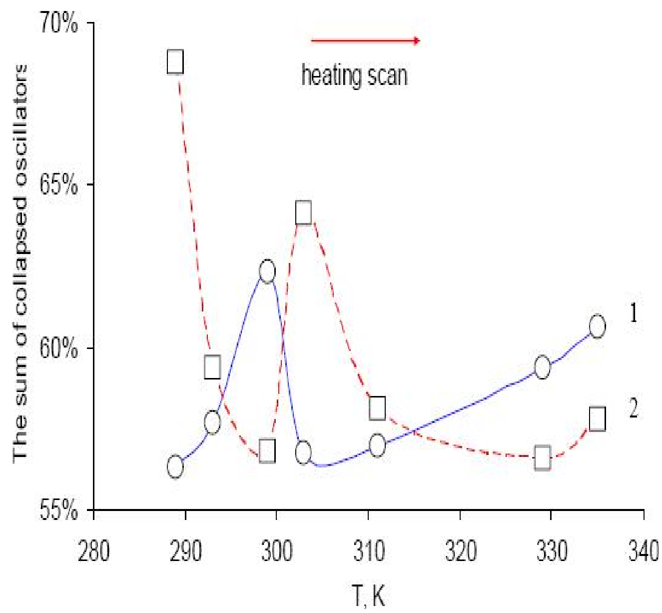


Figure 4 : Temperature dependence of the sum (Σ(abs(-*f*))) of collapsed oscillators in eggs. (0.2 Ê/min.). 1 – egg-white, 2 - egg yolk.

In Figure 4, the sum of collapsed clusters in free-range eggs versus temperature is given. The concentration of collapsed clusters in both eggs components changes differently at heating. At 300 K there is a maximum for egg-white since the maximum for egg yolk is shifted to 305 K, which could be caused by a large number of collapsed clusters at the temperature near the embryo formation (310 K). This high sum of collapsed clusters can be seen as a preliminary stage for the egg yolk protection from temperature fluctuations.

It would be of interest to investigate how the clusters behave in the eggs from battery hens. This possibility was given during the worldwide bird flu epidemic in 2005 when all hens were locked in (quarantine in North Germany). To compare the egg quality of free-range hens (June 2005) and battery hens (November 2005, after 45 quarantine days) a small group of isolated hens from a private hens grower (1 hen per m^2) was chosen. During the quarantine many less eggs have been laid, at the same feeding around 30-fold less. After the quarantine the eggs production was normal again. As shown in Figures 5 and 6 the ZGMS-spectra of egg yolk and egg-white are generally similar, however, they strongly differ from those of the free-range eggs (Figures 2 and 3). Furthermore, they are very near to the spectrum of 0.9 wt. % NaCl solution[6] containing SCIPs and to that one of egg yolk at 289 K (Figure 2). The SCIP signals are much stronger than those of the other oscillators and they overlap them, therefore. The super molecular structure in the egg yolk and egg-white has been strongly changed. This state was observed for free-range eggs at low temperatures only (Figure 2, 289 K) whereas it appears at all temperatures for the embryo origin in battery eggs to explained with an insufficient movement and stress of the hens. It stands out that for battery eggs there are signals in the area of high oscillating masses (Figures 5 and 6), which aren't available for free-range eggs. This could be an indication for that the battery eggs are structured not only in the area of small seed crystals but also in that one of large polymers probably, at the level of protein associates and of large SCIPs[15]. Some differences are observed for expanded clusters, in the spectra they are marked with an arrow. To our opinion these signals can only partially be assigned to SCIPs they rather characterize protein oscillators. Unlike free-range eggs the SCIP oscillations don't disappear at heating (Figures 5 and 6). Therefore, the embryo growth after the nano emulsion destruction in egg yolk starts according to different scenarios of protein-SCIPs' interaction which influences the eggs' quality. The stable presence of SCIPs in eggs could be caused by stress and hypo dynamics during the quarantine, that led to a salt enrichment in eggs although there aren't any differences between free-range and battery eggs visually.

As shown in Figure 6 at heating battery egg-white, changes in the ZGMS-spectra are little where the signals of the small water clusters $(H_2O)_{11\pm1}$ and $(H_2O)_{100}$ remain stable. In contrary to free-range eggs the super molecular structure in egg-white has lost its temperature dependence, it is dominated by SCIP signals. The "island" of cluster signals (indicated with arrow), moves to high masses as a result of association and coagulation processes at warming, probably.

The ZGMS-spectra of egg-white from free-range

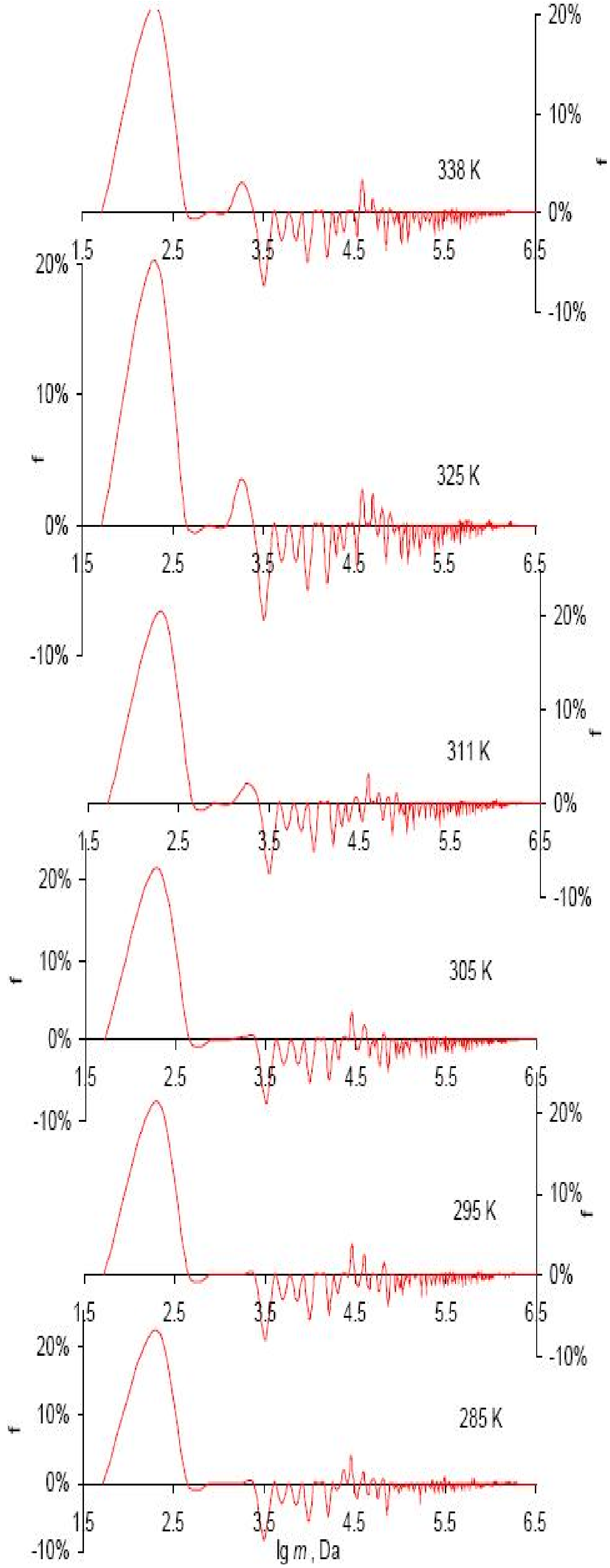


Figure 5 : ZGMS-spectra of egg yolk at different temperatures after 45 ± 3 quarantine days.

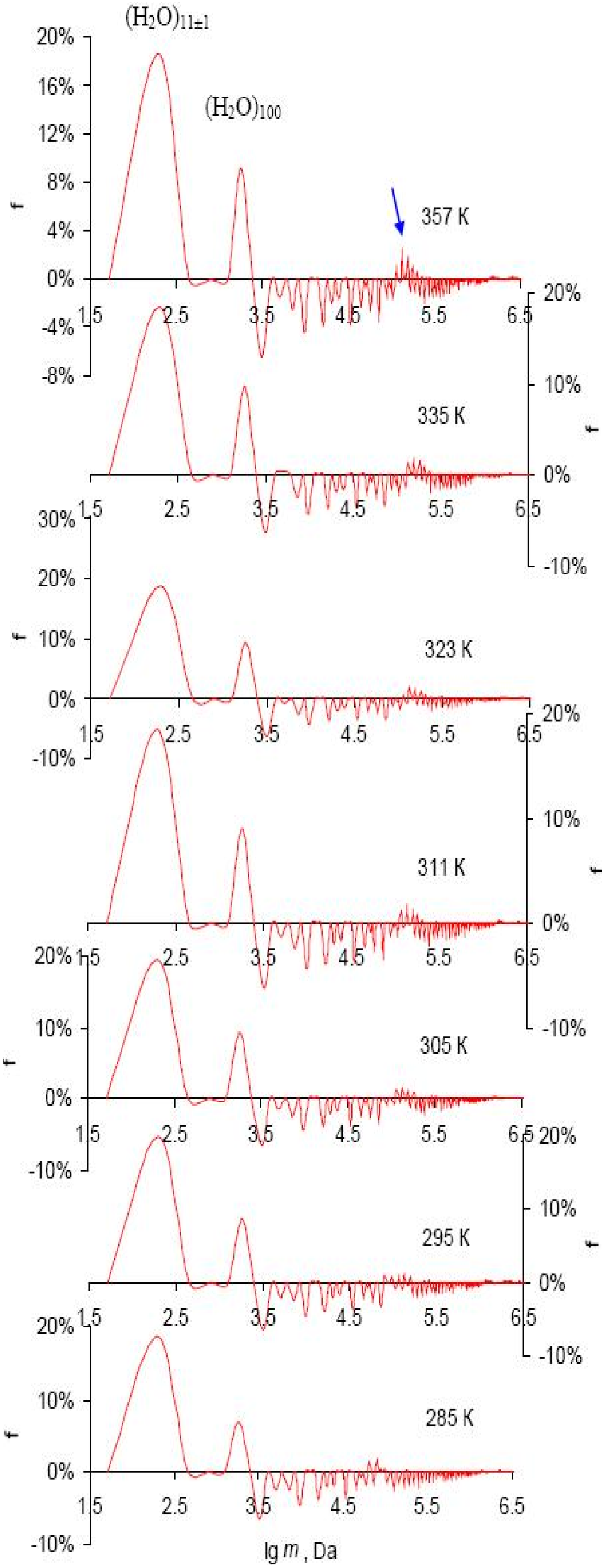


Figure 6 : ZGMS-spectra of egg-white at different temperatures after 45 quarantine days.

and battery eggs given in the Figures 3 and 6 are strongly different from each other. The detailed understanding of these differences could be the object of a special investigation. The stress factors of the quarantine therefore influenced the super molecular structure formation in egg-white and finally the egg quality generally. Psychological pressure, lack of light, room, movement and quiet are the main stress factors that led to an increased production of stress hormones in the hens´ body and accelerate ageing and destruction processes of biomatrices. Possibly, these hormones prevent the participation of SCIPs in biochemical processes of the egg-white synthesis and embryo growth.

The change of collapsed clusters in eggs after 1.5 month quarantine is shown in Figure 7. At warming, the concentration of collapsed clusters in egg-white reduced monotonously however with a little increasing in the temperature interval 305…315 K. This behavior is strongly different from that one of free-range eggs (Figure 4). The biomatrix super molecular structures were concluded to be strongly different in free-range and battery eggs, therefore. These differences should be explained with that hens in small closed rooms are permanently under psychological stress.

In the Figures 8 and 9 the ZGMS-spectra of the clusters' signal ratios in egg yolk and egg-white of free-

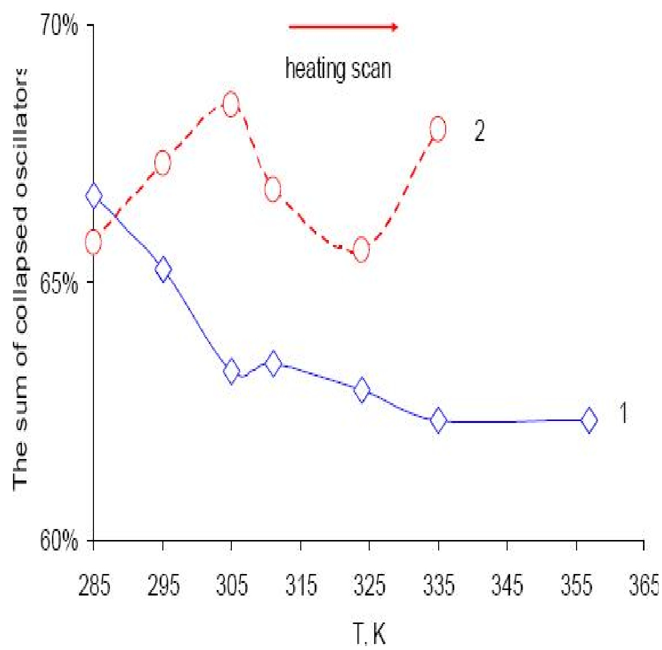


Figure 7 : Temperature dependence of the sum of collapsed oscillators (Σ(abs(-*f*))) in egg- white (1) and egg yolk (2) during the quarantine in North Germany in 2005. 0.2 K/min.

range and battery eggs are given, the absolute signal intensities of Figure 3 were divided by those from Figure 6 at the same temperature for what. The same procedure was made for the signal intensities of the Figures 2 and 5. By these measures, it was more simply to analyze the changes in the liquid structure at the level of oscillating clusters, domains, protein coils and their associates in addition, it is possible to see how these signals develops at heating. Large oscillators changed most strongly; concerning both coils and their associates. Comparing the signal intensities of free-range and battery eggs it was found that they are for the first ones considerably higher. From the other side it is known, that the signal intensity primarily depends on the interaction of an oscillator with the surroundings and then on the oscillator concentration[16]. The high signal intensity in free-range eggs is therefore described to a weaker interaction of the coils and their associates with the surroundings which is only possible when protein coils are ordered in a more dense structure. With temperature increase from 287 to 311 K (Figure 8) the signal ratio is reduced from 40…70 to 10…15, however, it rises up to 100 above 311 K. At 311 K the highest number of signals was measured, that describe the differences between the two egg types. The oscillator signal with the mass of 5.3 kDa is ascribed to the main domain in lysozym[17], but those with 153 and 371 kDa to the conalbumin dimer (76 kDa[12]) and ovalbumin octamer (44.5 kDa[12]), accordingly. The oscillator with the mass of 1520 kDa also seems to be part of the associate of protein coils, which decomposes at heating.

The strongest changes take place in egg yolk (Figure 9) where the signal ratios can achieve 200. The oscillations with masses of 390 kDa should belong to lipovitelline dimers (400 kDa[12]) they disappear as oscillators at 287 and 295 K and appear at 311 K.

In the following we want to analyze the oscillations of ovalbumin in egg-white (see Table), that appears as a monomer with different water contents: 6.3 wt. % for free-range eggs and 1.5 wt. % for battery eggs (Figure 9). Additionally, ovalbumin was observed as octamers too. The first octamer type, containing 4.3 wt. % water (expanded oscillator), was found for both egg types whereas the last one with 9.2 wt. % water (collapsed oscillator) for free-range eggs only (Figure 10). The water concentration in ovalbumin was determined by

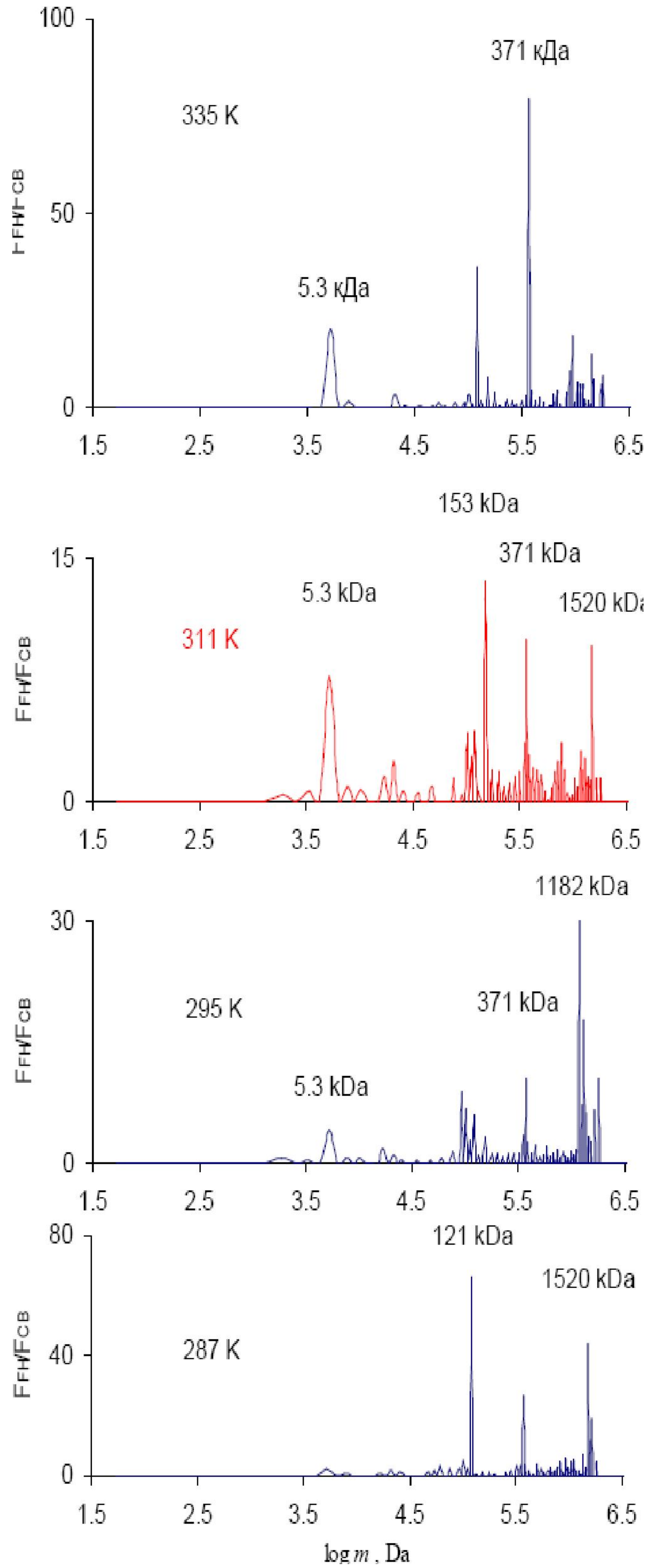


Figure 8 : ZGMS-spectra comparing the oscillators in egg-white (free-range eggs) at different temperatures. F_{FH}/F_{CB} - the ratio of oscillator signals in free-range eggs (F_{FH}= abs(f_{FH})) to those in battery eggs (F_{CB}= abs(f_{CB})). Oscillators with the same mass were compared.

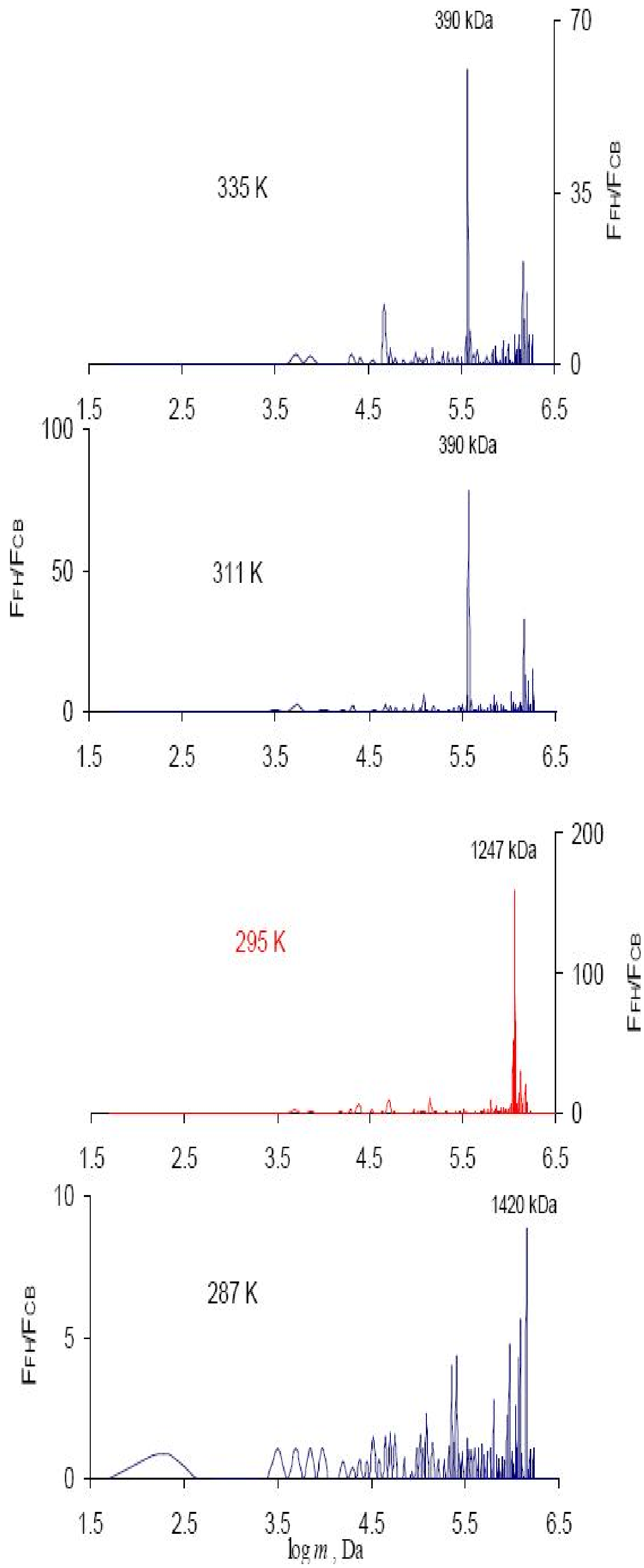


Figure 9 : ZGMS-spectra comparing the oscillators in egg yolk (free-range eggs) at different temperatures. F_{FH}/F_{CB} - the ratio of oscillator signals in free-range eggs (F_{FH} = abs(f_{FH})) to those in battery eggs (F_{CB} = abs(f_{CB})). Oscillators with the same mass were compared.

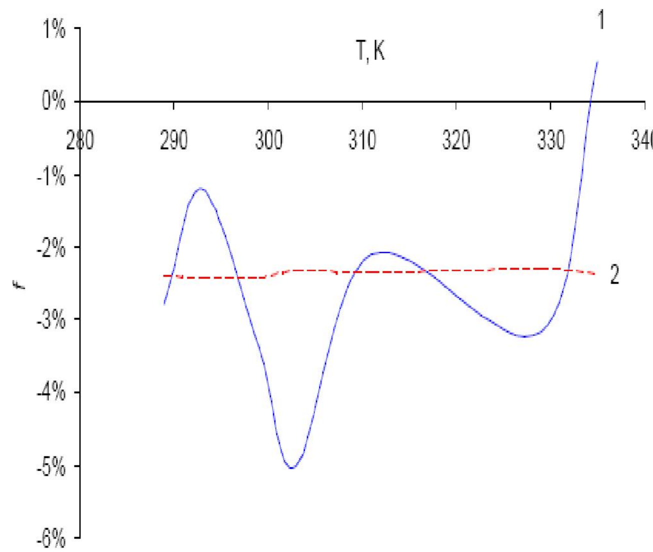


Figure 10 : Temperature dependence of the oscillations caused by ovalbumin monomers (*f*) in egg-white. 1 - free-range eggs (6.3 wt. % water), 2 – battery eggs (1.5 wt. % water).

subtracting the mass for ovalbumin (44.5 kDa[12]) from the experimentally observed oscillating mass.

As visible in Figure 10 the oscillating ovalbumin monomers in free-range eggs actively interact with the surroundings whereas those in battery eggs are in a “frozen” state. The loss of elasticity in the interaction with the surroundings could be an indication for a permanent stress the hens exposed. At 305 K the ovalbumin monomer is the most mobile in its vibrations and at this temperature a minimum is available in the curve the monomer coils are in the collapsed form with a minimal interaction with the surroundings.

Differently from free-range eggs in battery eggs there was found only one octamer type, which could be caused by permanent stress the hens exposed (Figure 11). The octamers in battery eggs are similarly to monomers in a “frozen” state (ordered SCIPs shell like water on the protein surface[18], scheme), those interaction with the surroundings is constant. This surroundings of the octamer prevents its normal interaction with the neighbours or the octamer is now surrounded by other neighbours than usual, perhaps. The lower water content in the ovalbumin coils of battery eggs in comparison with that one of free-range eggs (Table) and the large number of SCIPs (Figure 6) could give an answer on the monomer surroundings. Thus, the ovalbumin coils are in egg-white (battery eggs) in a cage that is formed by SCIPs. SCIPs strongly limit the oscillation possibilities of coils and their associates and consequently their interaction with the surroundings.

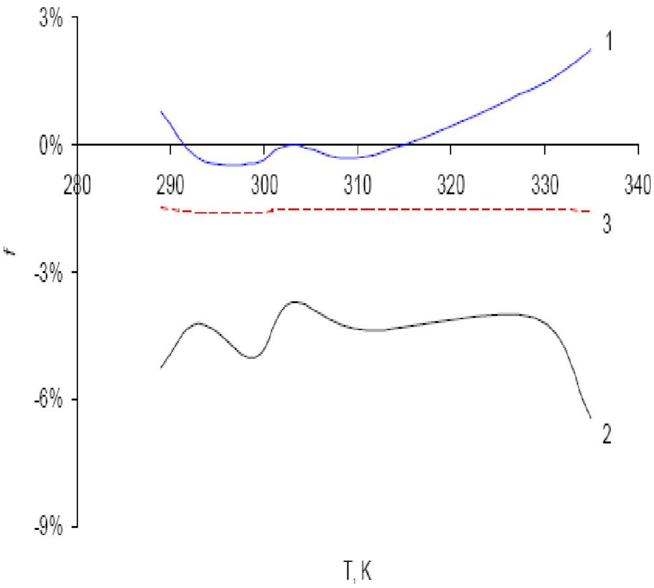


Figure 11 : Temperature dependence of the oscillations caused by ovalbumin octamers (*f*) in egg-white from free-range eggs: 4.3 wt. % water (1) and 9.2 wt. % water (2). For comparison battery eggs with 1.5 wt. % water (3).

Oscillations of ovalbumin dimers and tetramers were also found in the ZGMS-spectra (Table), possible structures of ovalbumin with the surroundings for both egg types shall be given in Figure 12. According to this model the ovalbumin coils from battery eggs are a little compressed and smaller compared with those of free-range eggs.

The oscillations of monomers and their associates are limited by the surface of the surroundings and by an interaction with surfaces fragments (Figures 1 and 12) and they can be identified with the ZGMS-spectroscopy too. For both egg types, the following models concerning the state of the coils and their interaction with the surroundings have been suggested (Figure 12).

TABLE : The content of included water (wt. %) in ovalbumin coils.

Oscillator (ovalbumin)	free-range eggs	battery eggs
	289 K	285 K
monomer	6.3	8.5
dimer	4.1	4.3*
tetramer	0	1.7
octamer I	4.3	4.3
octamer II	9.2	-

*For the dimers the signals lie thick besides each other.

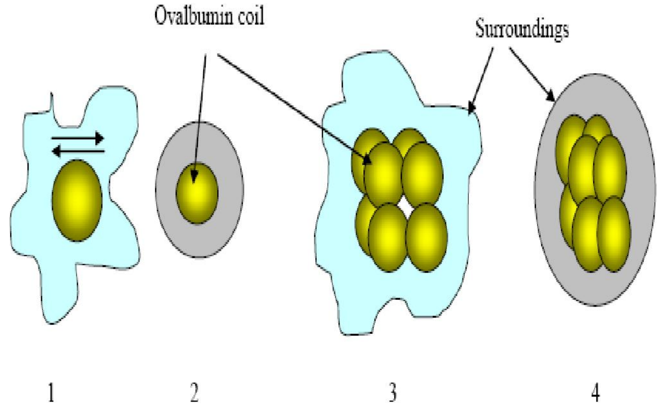
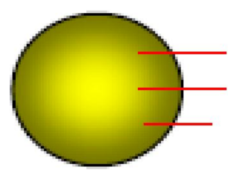


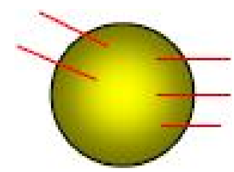
Figure 12 : Models describing ovalbumin coils and their interaction with the surroundings in egg-white. 1, 2 monomers, 3, 4 octamers. 1, 3 – free-range eggs, 2, 4 - battery eggs. The ordered shell of SCIPs is shown in grey.

Differently from the ovalbumin dimers of free-range eggs those of battery eggs show a wide spectrum of dimer signals with almost the same masses.

Based on the data of the table and Figure 12 the following models for the formation of protein associates can be represented.

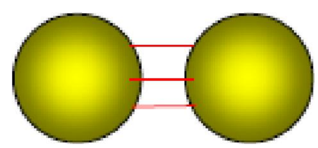


Monomer in free-range eggs (6.3 wt. % included water)

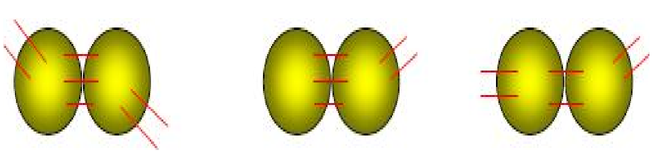


monomer in battery eggs (8.5 wt. % included water)

The ovalbumin coils with asymmetrical hydrophilic groups are solvated with water molecules (drawn as lines) where the hydrophilic groups are localised on the protein surface (ordered shell[18]).

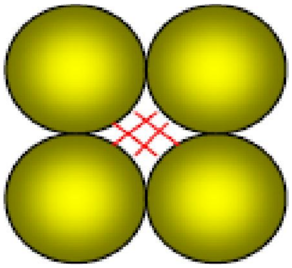


Model of an ovalbumin dimer from free-range eggs (4.1 wt. % included water)



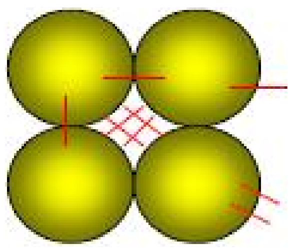
Models of ovalbumin dimers from battery eggs (4.3 wt. % included water)

Both the water content and the number of possible combinations for ovalbumin dimers from battery eggs should be higher around something according to this model.



Ovalbumin tetramer model from free-range eggs (0 wt. % included water)

To this model, the surface hydrophilic groups of the coils interact with themselves only and the tetramer is “dry”, therefore.



Ovalbumin tetramer model from battery eggs (1.7 wt. % included water)

For this ovalbumin tetramer from battery eggs, the hydrophilic groups are partial unordered and the tetramer is no longer “dry”.

Stress for hens was therefore found to change the coils’ conformation and super molecular structure in the biomatrices as well as to change the surroundings of the protein coils and their interaction with neighbour molecules.

CONCLUSIONS

The ZGMS method can be applied for fast controlling (0.5…30 s) the eggs´ quality at the level of the super molecular structure in egg yolk and egg-white biomatrices. Stress and captive breeding destructed the biomatrices of eggs at the level of cluster ensembles, changed the surroundings of proteins and protein conformations. The obtained results also would be interesting for the solution of other problems for example, for the development of bio-cosmic technologies for space flights and space stations.

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REFERENCES

[1] V.A.Zubow; Brauwelt, **27**, 795-796 **(2005)**.
[2] V.A.Zubow; Deutsche Milchwirtschaft, **21**, 926-927 **(2005)**.
[3] E.Dan Dudgeon, M.Russel Mersereau; Multidimensional Digital Signal Processing, Prentice-Hall, Inc., Englewood Cliffs, **(1984)**.
[4] K.V.Zubow, A.V.Zubow, V.A.Zubow; In Advances in Chemistry Research, Editor J.C.Taylor. Novapublisher. NY, in print, **5**, (2010).
[5] K.V.Zubow, A.V.Zubow, V.A.Zubow; J.of Biophysical Chem., In Print, **(2010)**.
[6] K.Zubow, A.Zubow, V.Zubow; Journal of Applied. Spectroscopy, (in Russian), **72(6)**, 766-772 **(2005)**.
[7] K.Zubow, A.Zubow, V.Zubow; Journal of Applied. Spectroscopy, (in Russian), **72(3)**, 321-328 **(2005)**.
[8] K.Zubow, A.V.Zubow, V.A.Zubow; J.of Modern Physics, In Print, **(2010)**.
[9] Yusuke Yamauchi, Hiromi Nakai; J.Chem.Phys., **123**, 034101-1- 034101-9 **(2005)**.
[10] S.Maheshwary, N.Patel, N.Sathyamurthy, A.D.Kulkarni, S.R.Gadre; The Journal of Physics Chemistry A., **105**, 10525-10537 **(2001)**.
[11] K.V.Zubow, A.V.Zubow, V.A.Zubow; Diagnostics of Materials, (in Russian), **74(9)**, 40-45 **(2008)**.
[12] H.D.Belitz, W.Grosch; Lehrbuch der Lebensmittel, Dritte, Uberarbeitete Auflage, S.439, Springer-Verlag, Berlin, Heidelberg, NY, Paris, Tokio, **(1987)**.
[13] A.Lenz, L.Ojamae; Chemical Physics Letters, **418**, 361-367 **(2006)**.
[14] K.V.Zubow, A.V.Zubow, V.A.Zubow; “Principles of Gravitation Spectroscopy, New Form of Molecular Matter, Processes. Fields”. Aist Handels- und Consulting GmbH, Dep.R&D, Berlin, Electronic Book (www.zubow.de), (in Russian)., 854 **(2010)**.
[15] Yannis, Kierzek, M.Andrzej, Saenger, Wolfram; The Journal of Physical Chemistry, **104(15)**, 3405-3406 **(2000)**.
[16] R.S.Drago; Physical Methods in Chemistry, Philadelphia, London, Toronto, W.B.Saunders Company, **(1977)**.
[17] M.Rafaee, T.Tezuka, K.Akasaka, M.Williamson; Journal of Molecular Biology, **327**, 857-861 **(2003)**.
[18] A.Shiryaev, D.L.Pagan, J.D.Gunton; The Journal of Chemical Physics, **122**, 234911 (0-10) **(2005)**.