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PHYSICOCHEMICAL STUDIES OF SYSTEMS AND PROCESSES

Use of Piezosensors for Determining the Composition of the Equilibrium Gas Phase of Aqueous Protein Solutions

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Abstract—Possibility of using modified piezosensors to determine the composition of multicomponent gas mixtures was studied. The sorption conditions of readily volatile components of aqueous protein solutions on modifier films of varied nature for piezoquartz resonators were examined and optimized. A set of methods for determining aroma-forming components of complex mixtures was developed. The possibility of using the methods developed for determining the nature of aroma-forming additives to milk drinks was demonstrated.

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The increasing impact of anthropogenic contaminants on the tropo-, hydro-, and atmosphere leads to severe negative changes in not only physical and moral, but also social health of humans and human society. The contamination of air, water, and soil and the possibility of their being severely damaged has become a frequent topic only recently; because of pronounced changes in technologies and formulations in food industry, the problem of safe food products is being openly discussed. Undoubtedly, toxic and poorly studied food components are the most hazardous to the human health.

According to the International Health Organization, 80–90% of substances foreign to humans comes with foodstuff, 4–7%, with water, and an even lesser amount, with air [1]. About 5% of samples of domestic products fail to satisfy hygienic regulations in their sanitary-chemical parameters [1].

Contamination of food raw materials is possible via introduction of artificial additives (aromatizers, dyes, stabilizers, emulsifiers), by metabolites of microorganisms used in manufacture of food, and by anthropogenic substances via soil, water, and raw materials. It is known that the same substances are differently distributed among the main matrix of food or nonfood nature and the gas phase and thereby determine numerous quality and safety parameters of finished products (emission level of readily volatile components from plastics, aroma of food products). As a consequence, there is an increased interest in development of new methods for detecting trace amounts of readily volatile components in complex mixtures, including protein matrices.

The aim of the study was to develop a set of methods for determining microscopic concentrations of alcohols, aldehydes, ketones, carboxylic acids in a gas phase that is in equilibrium with aqueous protein solutions by the piezoquartz microweighing.

As detecting elements served piezosensors in the form of AT-cut piezoquartz resonators (PQRs) with sensitive sorbent films on their electrodes.

In development of methods for determining how the composition of gas media changes, the following problems were to be solved: choice of the optimal modifier films for PQR electrodes, sensitive to the main readily volatile components of aqueous protein solutions and inert toward water vapor; modeling and fabrication of an array of piezosensors; development of an algorithm for formation of the overall response of the detector, processing of the experimental results (matrix of piezosensor responses), and deciding on the composition of the equilibrium gas phase (EGP); and development of a set of methods for determining trace amounts of aroma-forming components in complex mixtures (for the example of milk drinks).

EXPERIMENTAL

We performed the experiment on a MAG-8 static injector gas analyzer (SenTekh Limited-Liability Company, Voronezh) comprising a detector cell equipped with two pipes for input of a gas sample and for film regeneration and microprocessor for recording and processing of signals, connected to a computer and controlled by an original software serving to record piezosensor responses, perform metrological processing of the measurement results, and evaluate the adequacy of the overall analytical signals from the gas-analyzer ("visual imprints"). As sensitive elements were used bulk-acoustic-wave PQRs with a base oscillation frequency of 10 MHz (P'ezo Open Joint-Stock Company, Moscow). The PQR electrodes were modified by uniform deposition with a microsyringe of sorbents (concentration 5–10 mg cm^{-3}) by the "static drop evaporation" method. The optimal sorbents of analytically pure grade were chosen for each sorbent: ethanol, acetone, chloroform, toluene. The excess amount of a free solvent was removed from the film in a drying box at 40–45°C in the course of 30 min. The removal completeness of the solvents was judged from stability of oscillations of a PQR with a film. The optimal mass of the films was 10–15 μg.

We chose PQR electrode modifiers (sorbents) on the basis of the sensitivity criterion, using the previously formed database (furnished by piezoquartz microweighing, extractive concentration, and gas chromatography). We studied as electrode modifiers standard chromatographic phases with different polarities and specific sorbents: PEG-2000 polyethylene glycol, polyethylene glycol adipate (PEGA), polyethylene glycol sebacate (PEGSb), polyethylene glycol succinate (PEGSc), polyethylene glycol phthalate (PEGP), polyvinyl pyrrolidone (PVP), Triton Kh-100 (TKh-100), beeswax (BW), and also 4-aminoantipyrin (4-AAP), trioctylphosphine oxide (TOPO) and dicyclohexane-18-crown-6 (CrE) mixed with polystyrene (PS).

As objects of study were chosen animal proteins and their model solutions, and also protein matrices of natural origin. To verify the adequacy of the results of microimpurity determination, we chose food products containing a large amount of proteins and water (milk, milk drinks, drinking yogurts, cream) and aroma-forming additives of varied nature (of natural and artificial origin) [2]. Samples (2 cm^3) were placed in 20-cm^3 hermetically sealed weighing bottles with a polyurethane membrane on the cover, kept for 15 min to saturate the gas phase with readily volatile components, and 3-cm^3 samples of the EGP were taken. The EGP of aqueous protein solutions was sampled by the method of discrete gas extraction (heat-space analysis) [3]. An EGP sample was introduced with a gas syringe into the detection cell of the gas analyzer at a rate of 1 cm^3 s^{-1} and the oscillation frequency of each piezosensor in the array was recorded.

As the analytical signal of a piezosensor, ΔF (Hz), served the change in the oscillation frequency of a quartz plate as a result of sorption of readily volatile components from the gas phase onto the film of sorbents. The multidimensional signal of the array of piezosensors is the overall response formed by changes in the oscillation frequency of each element. A way for visualization of piezosensor responses in their exposure to gas mixtures is the kinetic visual imprint [4]. The integral kinetic visual imprint is constructed from signals of all the piezosensors, recorded a certain time (5, 10, 20, 30, 40, 60 s) after the beginning of vapor injection into the detection cell. A differential kinetic visual imprint is constructed by processing the integral kinetic visual imprints of EGP of a sample and matrix being tested by a special algorithm provided by the data processing software. All the measurements were made under identical conditions at a detection temperature of 20 ± 1°C. The detection cell and the piezosensors were regenerated by blowing with dried laboratory air for 15–20 min. The metrological processing of the results was performed by the standard procedure [5].

To verify the adequacy of the results obtained, model solutions were in parallel studied on a Tsvet Yauza liquid chromatograph (Khimavtomatika Research-and-Production Association, Moscow) by the HPLC method.[1] The chromatographic conditions were as follows: SS TESSEK SEPARON SGX column (l = 150 mm, d = 4 mm) packed with Silasorb C 18 sorbent of 5-μm fraction; ultraviolet detector (λ = 220 nm); eluent: 0.5% solution of orthophosphoric acid, acetonitrile (50 : 1, vol : vol), and twice-distilled water; eluent flow rate 1 cm^3 min^{-1}.

The metrological characteristics of our measurements are affected by the stability of operation of the electric circuit of RQR self-oscillations, quality of the selector layer on the piezosensor (thickness, viscosity, sorption mechanism, mass stability, resistance to natural interfering

[1] The study was performed at the Center of strategic research development, Voronezh State Technological Academy.

Table 1. Maximum responses of piezosensors with films of various sorbents in vapors of aqueous protein solutions

Sorbent	ΔF, Hz, at indicated volume fraction of water ω, vol %, in solutions					
	100	S_r, %	90	S_r, %	50	S_r, %
PEG-2000	70 ± 10	5.63	60 ± 6	4.18	32 ± 2	3.12
PVP	80 ± 23	10.53	70 ± 17	9.59	43 ± 14	11.13
PEGA	58 ± 9	7.85	47 ± 9	8.13	35 ± 7	9.25
PEGSc	62 ± 12	8.70	50 ± 11	9.05	34 ± 9	10.60
PEGSb	63 ± 14	8.37	52 ± 14	9.52	35 ± 8	9.63
PEGP	50 ± 11	8.92	43 ± 10	9.71	27 ± 7	9.74
TKh-100	42 ± 17	16.67	36 ± 7	8.33	23 ± 10	17.66
TOPO	17 ± 2	5.88	15 ± 2	4.71	12 ± 2	5.83
4-AAP	20 ± 3	5.26	17 ± 3	6.25	14 ± 2	7.69
CrE	24 ± 3	4.17	21 ± 3	4.76	18 ± 2	4.67
BW	6 ± 2	11.67	5 ± 2	14.14	5 ± 2	14.14

* S_r is the relative standard deviation.

Table 2. Maximum responses of piezosensors with films of various sorbents in vapors of model protein solutions (ω_{water} = 90 vol %) with addition of acetic acid, ethanol, acetone, and ethyl vanillin ($\omega_{additive}$ ~ 0.03 vol %)

Sorbent	ΔF, Hz							
	acetic acid	S_r, %	ethanol	S_r, %	acetone	S_r, %	ethyl vanillin	S_r, %
TOPO	18 ± 2	5.55	20 ± 3	6.12	23 ± 3	5.32	20 ± 2	3.53
4-AAP	20 ± 3	6.80	20 ± 2	5.56	22 ± 3	5.57	20 ± 2	5.00
CrE	28 ± 4	5.65	30 ± 5	7.07	32 ± 5	6.63	28 ± 4	5.65
BW	5 ± 2	14.14	5 ± 2	14.14	6 ± 2	11.67	6 ± 2	11.67

components), correctness of sample preparation and EGP sampling, and algorithm for recording of responses and formation of the overall signal.

A PQR self-oscillation circuit stable against external factors (temperature differences, vibrations) with a multistage stabilization of the feed voltage was developed. Application of circuits of this kind substantially improves the interference immunity of the signal measurement system.

We studied the distribution of water vapor, readily volatile acids (lactic, citric, propionic, acetic, formic, butyric, caproic, caprylic, capric, lauric), aldehydes (acetaldehyde), alcohols (ethanol), ketones (acetone) among the liquid phase of an aqueous protein solution and the EGP over the solution. The content of substances in the EGP was determined using high-sensitivity piezosensors with sorbents exhibiting selectivity toward the chosen classes of compounds.

In the first stage, we studied model systems based on an animal protein (milk proteins) and water (Table 1). The chosen sorbents were divided into three groups, depending on their sensitivity to water vapor. The first group included PEG-2000 and PVP as the most hydrophilic sorbents [6]. The sensitivities of microweighing with these films to water vapor are close (~0.8 Hz vol %$^{-1}$), which exceeds by more than 15% that with sensors with other sorbents. Piezosensors with films of this kind can be used to monitor the content of residual moisture in dehydrated protein matrices (e.g., cheeses, curds). The second group of sorbents includes polyethylene glycol esters: PEGA,

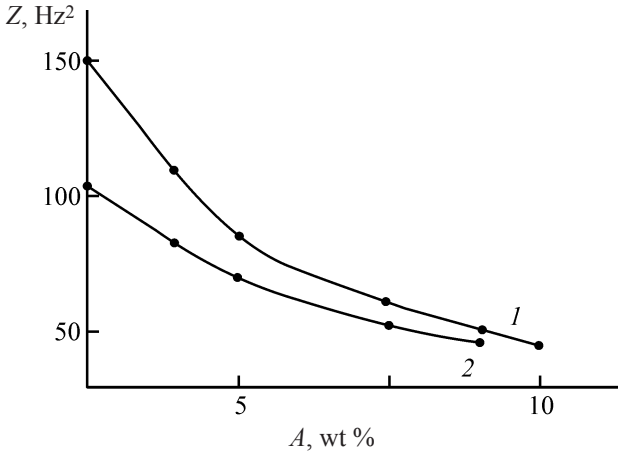

Fig. 1. Area of the overall multidimensional signal Z from an array of piezosensors vs. the mass fraction A of LS in model aqueous protein solutions with a volume fraction of water of (*1*) 90 and (*2*) 50 vol %.

PEGSc, PEGSb, PEGP, TKh-100. Water vapor is sorbed from the gas phase on these films in competition with readily volatile acids, aldehydes, alcohols, and ketones [6]. To diminish the influence of water and improve the determination selectivity, sorbents of the first and second groups were excluded from the piezosensor array. The third group included specific sorbents (TOPO, 4-AAP, BW, CrE) exhibiting the lowest sensitivity to water vapor. We studied on films of these sorbents the distribution of carboxylic acids (for the example of acetic acid), alcohols (ethanol), ketones (acetone), and ethyl vanillin (Table 2). For this purpose, we prepared model aqueous solutions (ω_{water} = 90 %) with addition of 0.03 vol % acetic acid, acetone, ethanol, and ethyl vanillin, which corresponds to their quantitative content determined by gas chromatography [7].

It was found that, with a piezosensor with a BW film, it is impossible to record insignificant changes in concentrations of readily volatile organic compounds in the EGP of aqueous protein solutions, because the analytical signals obtained in water vapor and model aqueous protein solutions with addition of 0.03 vol % acids, alcohols, ketones, and ethyl vanillin are statistically undistinguishable. Thus, all further studies were performed with piezosensors modified with films of TOPO, 4-AAP, and CrE mixed with PS. With a smaller number of piezosensors in the array, it is impossible to record significant changes in the composition of the EGP of aqueous protein solutions. Use of a larger number of piezosensors is inadvisable because this may lead to overloading of the kinetic visual imprint, which will impair its metrological reliability, hinder interpretation of the analytical signal as regards the EGP composition, and make longer the information processing.

In the next stage, we studied the influence exerted by the content of lipophilic surfactants (LS) in model

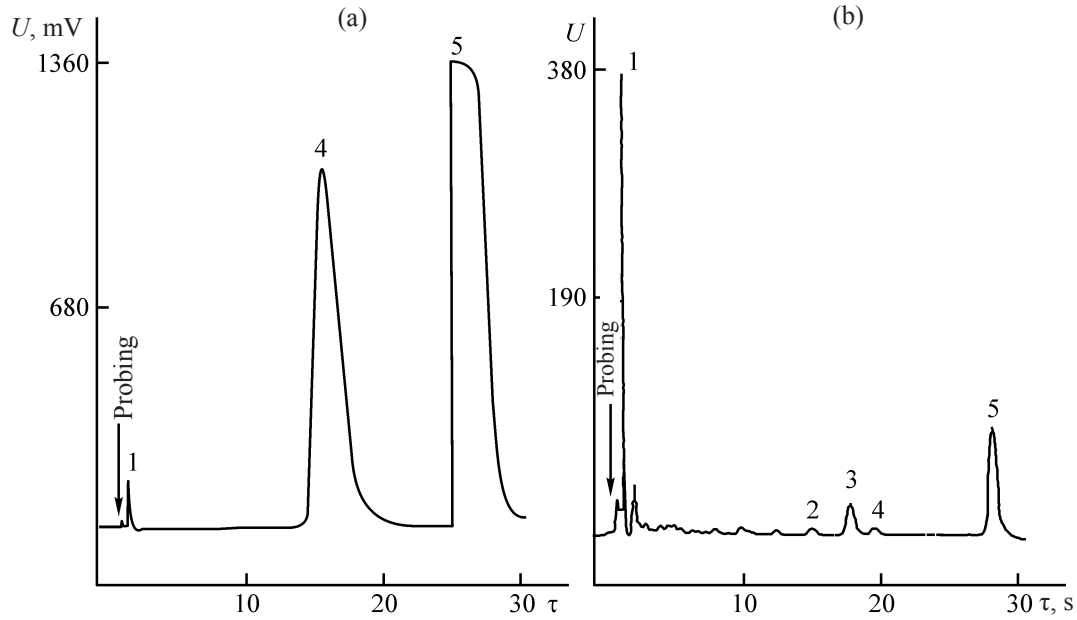

Fig. 2. Chromatograms of aqueous-alcoholic solutions of (a) artificial Vanillin aromatizer and (b) extract from natural vanilla flowers (*U*) Detector response and (τ) time.(1) Ethanol, (2) vanillic acid, (3) isovanillin, (4) ortho-vanillin, (5) ethyl vanillin; all other peaks correspond to vanillin derivatives.

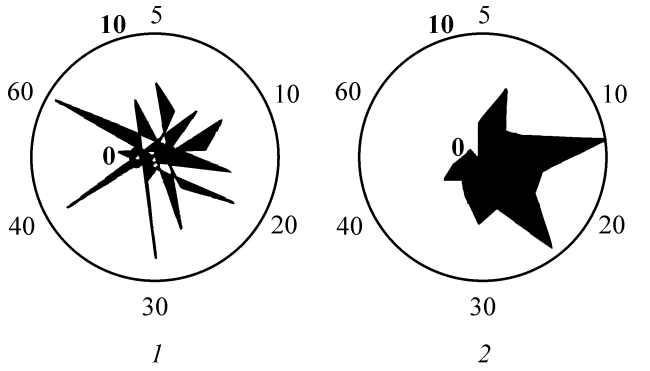

Fig. 3. Kinetic visual imprints of the EGP of (*1*) artificial Vanillin aromatizer and (*2*) extract from natural vanilla flowers. Digits around a circle, time in which piezosensor signals are recorded; the same for Figs. 4, 5.

aqueous protein solutions on the distribution of readily volatile acids, aldehydes, alcohols, and ketones among the liquid and gas phases (Fig. 1). It was found that, as the mass fraction of LS (fats) increases, the concentration of readily volatile substances in the gas phase becomes lower, which is confirmed by the decrease in the overall multidimensional signal from the array of sensors. This is attributed to a change of the hydrophilic-lipophilic properties of the protein matrix, which results in that the emission of readily volatile components into the EGP decreases. This pattern of distribution of readily volatile substances among the liquid and gas phases can be used to evaluate the fat content of high-protein food products (milk, drinking yogurts, milk drinks and cocktails).

In recent decades, the organoleptic parameters and, in particular, aroma of food and nonfood products (ink fillers, plastics, rubber articles for children, foodstuff) have been purposefully modified, to improve their consumer properties, by addition of flavoring formulations [8]. The quantitative content of flavoring substances in finished products is not always justified and controlled. In addition, different amounts of aroma-forming substances are introduced into substrates of varied nature to reach the same aroma level. Let us consider the distribution of the Vanillin aromatizer, most widely used in food and nonfood industries, among the liquid phase of an aqueous protein solution and the gas phase over this solution. The HPLC method revealed pronounced differences between the compositions of aqueous-alcoholic solutions ($\omega_{ethanol}$ = 25 vol 5, $\omega_{additive}$ = 0.03 vol %) of an extract from vanilla flowers and industrial Vanillin aromatizers (Poland, Germany) (Fig. 2). It was found that an aqueous-alcoholic solution of an extract from vanilla flowers contains, in addition to ethyl vanillin, *ortho*-vanillin, isovanillin, and

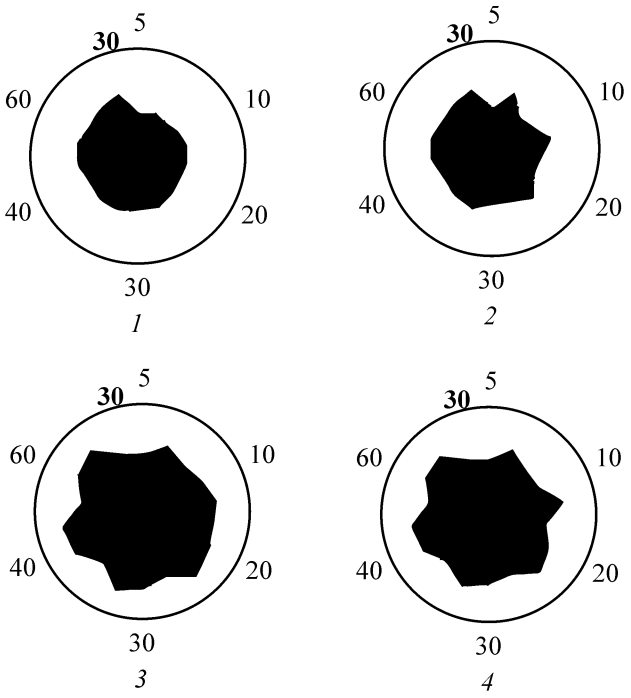

Fig. 4. Integral kinetic visual imprints of the EGP of model protein solutions (*1*) without additives and with addition of (*2*) extract from natural vanilla flowers, (*3*) artificial Vanillin aromatizer, and (*4*) mixture of natural vanilla and artificial Vanillin aromatizer.

their derivatives. Ethyl vanillin determines the vanilla odor of the extract, and its homologs and derivatives have only supplementary importance. The compositions of aqueous-alcoholic solutions of artificial Vanillin aromatizers are identical. In contrast to the natural raw material, the artificial aromatizer contains two components in large amounts. Because the compositions of vanilla additives are different, the distribution of their readily volatile components on the thin films of piezosensors will also probably be diverse.

Under identical conditions, we obtained kinetic visual imprints of the EGP of the artificial Vanillin aromatizer and an extract from natural vanilla flowers (Fig. 3). As follows from Fig. 3, the configurations of the kinetic visual imprints are markedly different and, consequently, the MAG-8 static injection gas analyzer can be used to determine the nature of an aroma-forming additive. However, the distribution of readily volatile substances among the liquid and gas phases changes upon introduction of aroma-forming additives into the protein matrix. To evaluate the interfering influence of aldehydes, ketones, alcohols, and carboxylic acids ($\omega_{additive}$ = 0.02 vol %) and water (ω = 90 vol %) in the

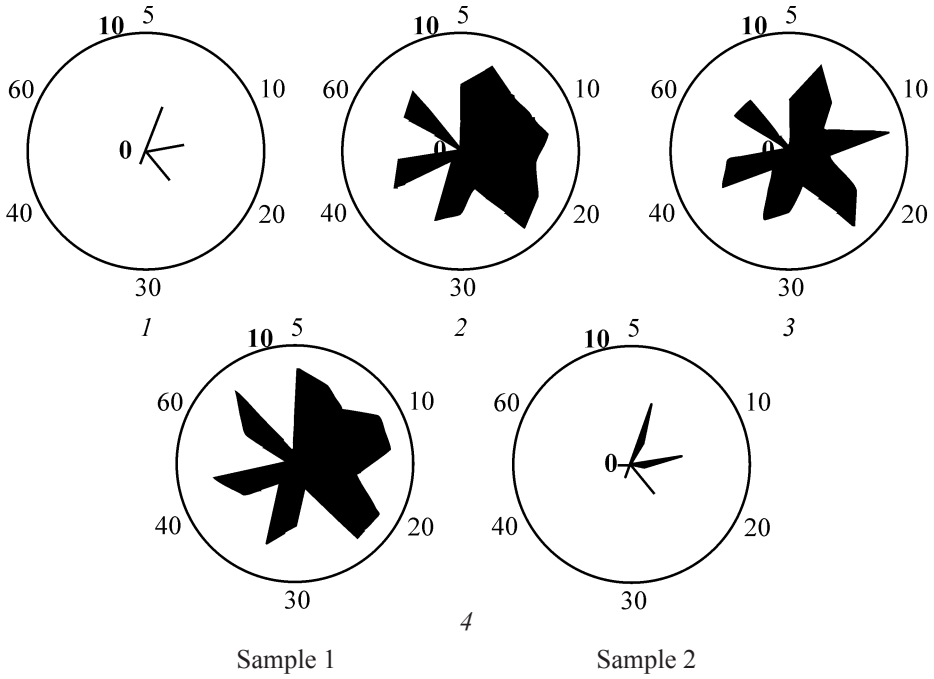

Fig. 5. Differential kinetic visual imprints of the EGP of model protein solutions with addition of (*1*) natural vanilla flowers, (*2*) artificial Vanillin aromatizer, (*3*) mixture of natural vanilla and artificial Vanillin aromatizer, and (*4*) milk drinks with vanilla (samples 1, 2).

protein matrix on the distribution of aroma-forming substances, we constructed integral kinetic visual imprints of the EGP of model aqueous protein solutions with addition of 0.02 vol % artificial Vanillin aromatizer and an extract from natural vanilla flowers (Fig. 4). It was found that the influence of the interfering components is significant and the nature of vanilla additives cannot be identified.

To solve the problem, we used a differential algorithm of piezosensor signal processing, which made it possible to minimize the effect of water, aldehydes, ketones, alcohols, and acids and to detect slight changes in the sample composition upon introduction of additives (Fig. 5). It was found that the nature of an additive strongly affects the configuration of the kinetic differential visual imprints. For example, addition of a natural raw material to the matrix results in that the overall signal from the array of piezosensor somewhat increases, compared with addition of a synthetic aromatizer. The resulting differential kinetic visual imprints of the EGP of model aqueous protein solutions with aroma-forming additives confirm the different types of sorption of readily volatile components on thin modifier films.

Based on the fundamental aspects of sorption of mixtures of readily volatile substances, found in this study, we developed a set of procedures for determining aroma-forming additives in the EGP of aqueous protein solutions in the presence of microscopic amounts of alcohols, aldehydes, ketones, and carboxylic acids. Application of the differential algorithm for processing of piezosensor signals enables detection of microscopic amounts of aroma-forming additives.

Let us demonstrate the possibility of using the set of procedures we developed to identification of the nature of aroma-forming substances in samples of milk cocktails with additions of vanilla. For this purpose, we obtained under identical conditions differential kinetic visual imprints of the EGP of the samples under study (Fig. 5, samples *1* and *2*). The mass fraction of lipids in all the samples was 2.5 wt %. The presence of artificial Vanillin aromatizer and its content ("normal," "excessive") in the milk drink samples studied is determined from the shape and area of the kinetic visual imprints as compared with the database evidence from analysis of reference samples (Fig. 5, *1*–*3*).

Differential kinetic visual imprints of the EGP of sample *2* and a protein mixture with addition of an extract from natural vanilla flowers are almost identical. No synthetic ingredients were found in this sample. Another milk drink (sample *1*) contains an additive with a chemical composition different from that of natural vanilla. In all probability, this additive is the artificial

Vanillin aromatizer. The overall error of measurements by an array of piezosensors does not exceed 25%.

CONCLUSIONS

(1) The number and nature of PQR electrode modifier films sensitive to the main readily volatile components of aqueous protein solutions and inert toward water vapor were optimized. An array of piezosensors was formed.

(2) An algorithm for forming an overall response of the detector and processing the experimental results (matrix of piezosensor responses), and deciding about the EGP composition was developed.

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