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Investigating the properties of egg white pasteurised by ultra-high hydrostatic pressure and gamma irradiation by evaluating their NIR spectra and chemosensor array sensor signal responses using different methods of qualitative analysis

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Abstract

Nowadays there is an increasing consumer demand for high quality, minimally processed, additive-free and microbiologically safe foods. The future implication of non-thermal food processing techniques, such as ultra-high hydrostatic pressure and gamma irradiation develop rapidly in the food industrial area. Being non-thermal treatments, components involved in the sensory and nutritional quality remain unaffected. In order to gain experience on feasibility of non-thermal pasteurisation of egg, the effects of ultra-high hydrostatic pressure (UHP) treatment and gamma irradiation levels were investigated. In the present study fresh liquid egg white samples were subjected to ultra-high hydrostatic pressure of 400 MPa for 15 min at 4 °C and gamma irradiation of 3 kGy doses. Near infrared spectroscopy and chemosensor array measurements (electronic nose) were performed in order to monitor the quality changes caused by the applied treatments compared to untreated control samples.

The recorded near infrared spectra and the sensor signal responses of the chemosensor array were classified by our self-developed qualitative evaluation method the polar qualification system (PQS) and the spectra recognition tool (SRT). The results of PQS were compared to traditional qualitative mathematical statistical methods such us principal component analysis (PCA), linear discriminant analysis (LDA) and to their combination using the first few principal components in calculation of the discriminant functions (PCA+CDA).

Keywords: NIR spectroscopy; Chemosensor array; Electronic nose; High hydrostatic pressure; Egg white

1. Introduction

Nowadays there is an increasing consumer demand for high quality, minimally processed, additive-free and microbiologically safe foods. The application of non-thermal food processing techniques, such as ultra-high hydrostatic pressure (**UHP**) and gamma irradiation develop rapidly in the food industrial area. The application of "ultra-high hydrostatic pressure" for food processing consists of subjecting the food to pressures in the range of 50–800 MPa. The UHP extends the shelf-life of the foods, inactivates the vegetative microorganisms, some enzymes, promotes the germination of bacterial spores into heat sensitive cell state, while retains vitamin content, preserves natural flavours. This new technology follows the "minimal processing" concept minimizing the quality degradation utilizing less energy. Irradiation is known and applied for decades in the range of 1–10 kGy for reducing bacterial contamination, sanitation and modification of food allergens.

Chicken egg albumen represents an extensively used food ingredient, mostly because of its functional properties. The gelling, emulsifying and foaming properties of fresh albumen are fundamental for making possible production and assessment of the final characteristics (texture, flavour, etc.) of many products [1].

Outbreaks of *Salmonella* enteritides can be traced back to consumption of raw or undercooked eggs contaminated internally with the bacteria. The only control measure against

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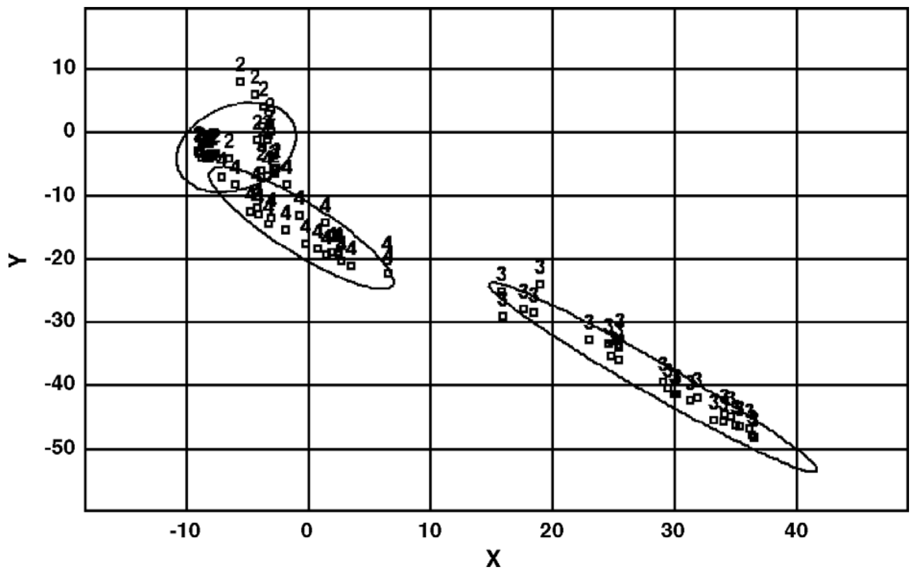

Fig. 1. The location of the quality points of the investigated egg white samples on the PQS quality plane using the optimum sequence of their electronic nose signal responses determined by automatic sequence optimisation. 2: control, 3: 3 kGy, 4: 400 MPa.

internal infection in shell eggs is pasteurisation. The U.S. minimum pasteurisation parameters of 60 °C for 3.5 min [2] have been reported to be sufficient. In contrast most of the functional properties of egg albumen are lost or modified after the mildest heat treatment because of the susceptibility of egg proteins to coagulation or thermal denaturation with the formation or destruction of covalent bonds [3]. It means alternative technologies must be developed and implemented considering sanitation and preservation of eggs. High-hydrostatic pressure and irradiation treatment are attractive alternatives to heat pasteurisation.

Presently it is well known that ultra-high pressure can be used to obtain safe foods with identical characteristics to fresh products. With this pressure technology (at low or room temperature) egg producers may be able to improve the microbiological quality of egg products without impairing their functional properties. Since pressure is transmitted instantaneously and homogeneously throughout all food it does not affect the covalent bonds or produce overtreated zones as may occur in the thermal treatment [4,5]. The conformation of the main protein component of egg white, ovalbumin, remains fairly stable when pressurised at 400 MPa; this may be due to the four disulfide bonds and the non-covalent interactions stabilizing the three-dimensional structure of ovalbumin. Meanwhile, it was observed by some authors that liquid egg white partially coagulated when treated at pressure >500 MPa [6].

Study by Ma et al. [7] showed irradiation of eggs with 2–4 kGy which was effective in pasteurisation of liquid and frozen egg products and according to Tellez et al. [8] 2 or 3

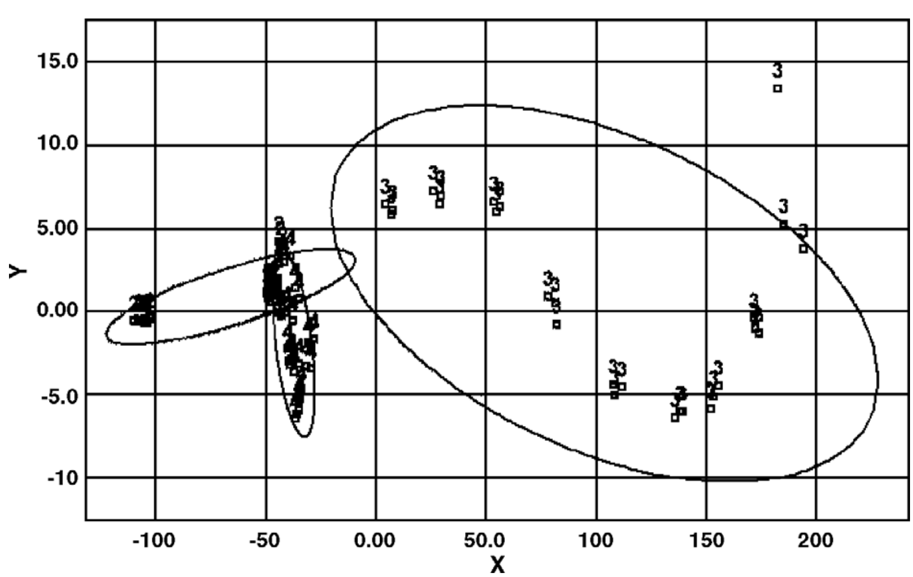

Fig. 2. The principal component analysis score plot of the electronic nose data of the investigated egg white samples on the projection plane determined by PC1–PC2. 2: control, 3: 3 kGy, 4: 400 MPa.

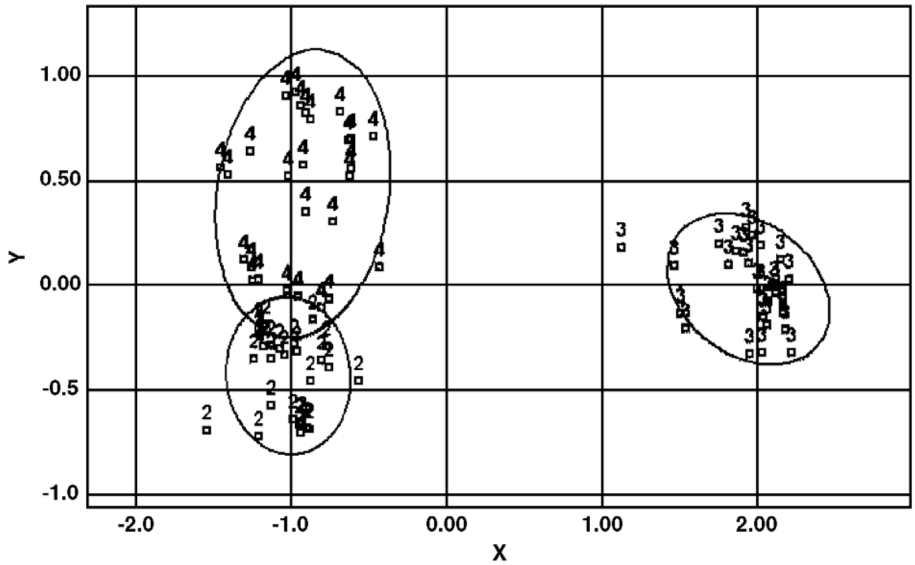

Fig. 3. The discriminant analysis score plot of the investigated egg white samples. The discriminant functions were calculated using the original sensor signal response of the electronic nose. 2: control, 3: 3 kGy, 4: 400 MPa.

kGy reduced bacterial contamination to non-detectable levels in eggs. Since 2000, the Food and Drug Administration (FDA) approved the use of up to 3 kGy ionising radiation dose to reduce the level of *Salmonella* in shell eggs (FDA Fed Reg 65, 2000). Moreover structural modification of food allergens by gamma irradiation was recently observed in several researches, which is of great importance, since egg is one of the most allergenic food, and several results have indicated that ionising radiation could reduce allergenicity or antigenicity by the destruction of the binding epitopes of food protein [9,10]. In order to gain experience on feasibility of non-thermal pasteurisation of egg, the effects of ultra-high hydrostatic pressure (UHP) and gamma irradiation treatments were investigated. In the present study fresh liquid egg white samples were subjected to high hydrostatic pressure and gamma irradiation subsequently. Near infrared spectroscopy and chemosensor array measurements (electronic nose) were performed in order to monitor the quality changes caused by the applied treatments compared to untreated control samples.

2. Materials and methods

Fresh liquid egg white samples were subjected to high hydrostatic pressure of 400 MPa for 15 min at 4 °C and gamma irradiation of 3 kGy doses subsequently. The samples were measured right after the treatments on an "electronic nose" and on a near infrared spectrometer in order to see the differences in

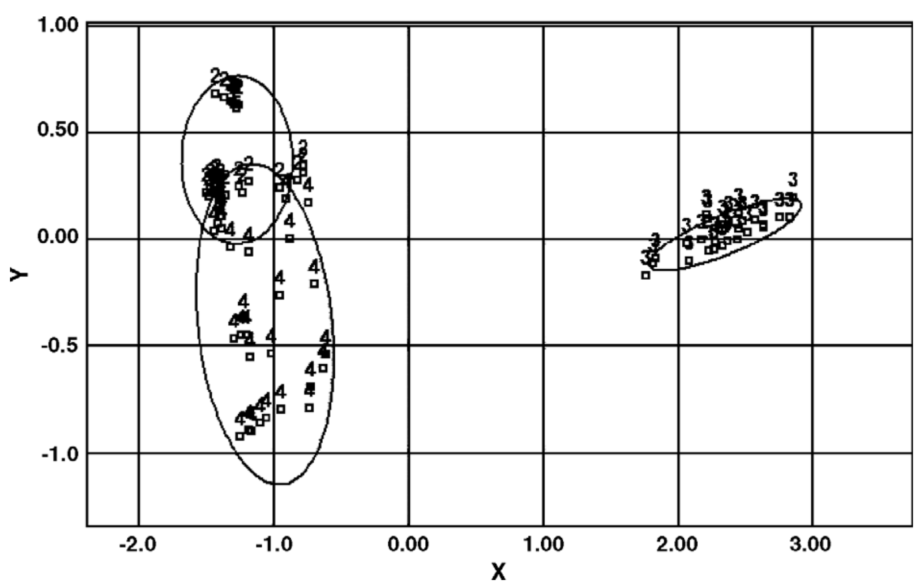

Fig. 4. The discriminant analysis score plot of the investigated egg white samples. The discriminant functions were calculated using the first 6 principal components of the sensor signal response. 2: control, 3: 3 kGy, 4: 400 MPa.

Table 1
The numerical comparison of the applied methods for electronic nose data using the sensitivity values (top) and the percent of correct classification in CDA cross validation (bottom)

	PQS	PCA	CDA	PCA+CDA
Control–irradiated	3.79	1.90	6.40	5.10
Control–UHP	1.41	0.88	1.09	1.24
Irradiated–UHP	2.49	2.11	4.67	3.87

	PQS	PCA	CDA	PCA+CDA
Originally grouped cases	92.8%	84.0%	92.6%	91.4%
Cross validated cases	92.6%	84.0%	91.4%	91.4%

odour and in the near infrared properties caused by the various treatments for extending the shelf-life. The near infrared spectra were recorded on a MetriNIR 10–17 PR type NIR spectrometer in the wavelength range of 1000–1700 nm with a spectral step of 2 nm. An AS 3320 type electronic nose, produced by AppliedSensor AG, was used during our measurements having chemosensor array consisting of 23 sensor elements. “Electronic nose” is a generic name for an analytical instrument that contains an array of chemical sensors (chemosensor array) whose outputs are integrated by advanced signal processing to identify complex odour mixtures. For evaluation first Polar Qualification System (PQS) [11] was applied using automatic sequence and automatic wavelength range optimisation for the sensor signal responses and for the near infrared spectra of the samples. Then for evaluation also Sample Recognition Tool (SRT) was used to discriminate the samples and to produce the classification (confusion) matrixes. To compare the results of PQS and SRT with the classical mathematical methods principal component analysis (PCA), canonical discriminant analysis (CDA) and their combination (PCA+CDA) were performed calculating the discriminant functions using the first few principal components.

3. Results and discussion

The location of the quality points of the investigated egg white samples can be seen in Fig. 1 on the PQS quality plane using the optimum sequence of the electronic nose signals. Although the quality points of the UHP treated samples are close to the quality points of the control samples as it is shown in Fig. 1 representing the PQS quality points (centres of the electronic nose sensor signal responses represented as polar spectra in the optimal data sequence) of the investigated sample sets, the differently treated egg white samples could be clearly distinguished. As it can be seen the irradiation of 3 kGy doses causes bigger difference (higher distance) between the quality points of the control and the irradiated samples than the difference between the control and the pressurised samples. This means that compared to the UHP treatment the irradiation of the investigated egg white causes more changes in the volatile components around the sample detectable by a chemosensor array instrument. Assuming interaction between this difference in the detectable volatiles and the human sense of smell based on this result it can be concluded that the UHP treatment preserves the smell of the samples. The results of the PQS evaluation were compared with principal component analysis and with discriminant analysis. Fig. 2 shows principal component analysis score plot of the same sample sets using the projection plane determined by the first and the second principal components. Here the clusters are a bit overlapped, the separation is not as good as the PQS method. In Fig. 3 the result of discriminant analysis can be seen where the discriminant functions were determined using original sensor signal responses coming directly from the instrument (23 data), while Fig. 4 shows the scores where the discriminant functions are derived from the first 6 principal components determined by principal component analysis. As it can be seen using the traditional methods the clusters are a bit overlapped, the

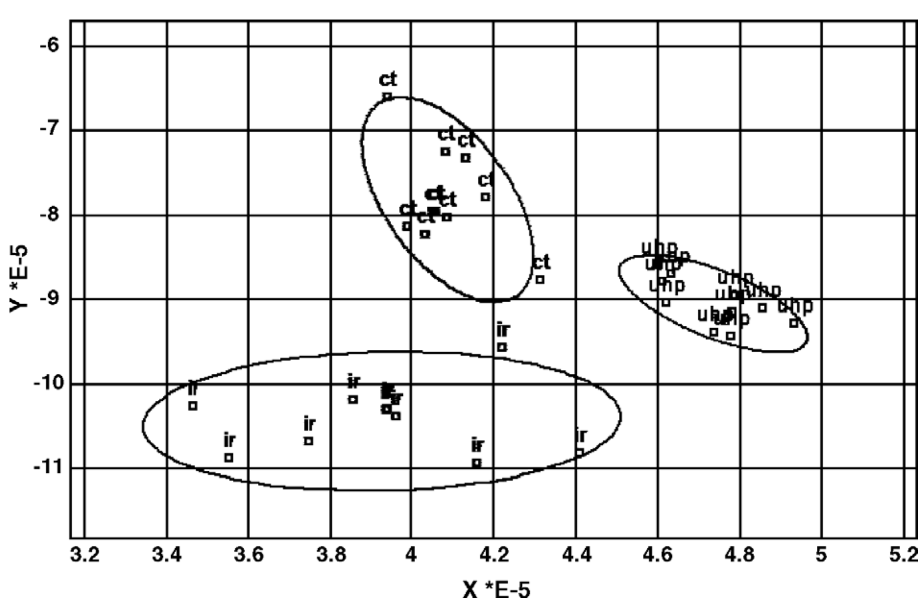

Fig. 5. The quality points of the investigated egg white samples on the PQS quality plane using the optimum range of the 2nd derivative NIR spectra determined by wavelength range optimisation. ct: control, ir: 3 kGy, UHP: 400 MPa.

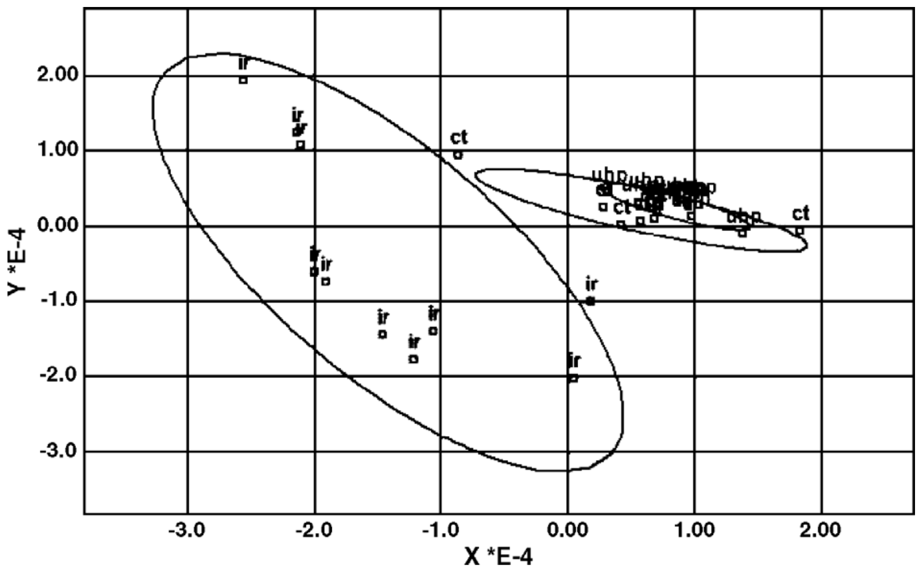

Fig. 6. The principal component analysis score plot of the investigated egg white samples using the 2nd derivative of their NIR spectra on the projection plane determined by PC1–PC2. ct: control, ir: 3 kGy, UHP: 400 MPa.

separation is not as good as the PQS method. The clusters belonging to the same sample sets are surrounded by two standard deviation ellipses. Besides the graphical comparison presented in Figs. 1–4 the PCA and the CDA scores were imported into the PQS software and the sensitivity values among the investigated groups were calculated in each classification method expressing the effectiveness of the classification numerically. Table 1 introduces these sensitivity values (S) among the investigated groups. In the case of $S=2$ the ellipses of the two standard deviations touch each other. Table 1 shows sensitivity values among the different sample sets using different evaluation methods for comparison (top), in the bottom of the table the percentages of the correctly classified samples are given for the different methods, where the value of the sensitivity $S = D\mathrm{abs}/(s_1+s_2)$, where D_{abs} is the distance between the centre of clusters of the quality points, s_1 and s_2 are the standard deviation of the quality points belonging to the separate clusters. Looking at the top of Table 1 it can be seen that the S values – showing the separability – are different for the different sample set pairs using electronic nose. The CDA method gives the best separation between control and irradiated samples, while PCA+CDA combination provides a bit better separation between control and pressurised samples. PQS method provides the best separation between control and pressurised samples , and the separation between control and irradiated samples is also very good. PCA method proved to be the poorest separation method in this particular case. Looking at the bottom of Table 1 in which the cross

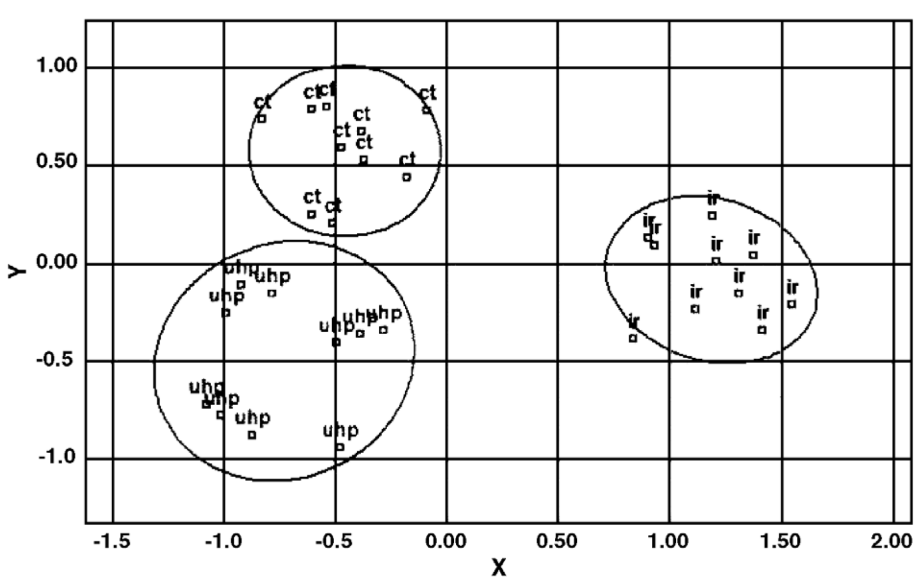

Fig. 7. The discriminant analysis score plots of the investigated egg white samples. The discriminant functions were calculated using the 2nd derivative spectra. ct: control, ir: 3 kGy, UHP: 400 MPa.

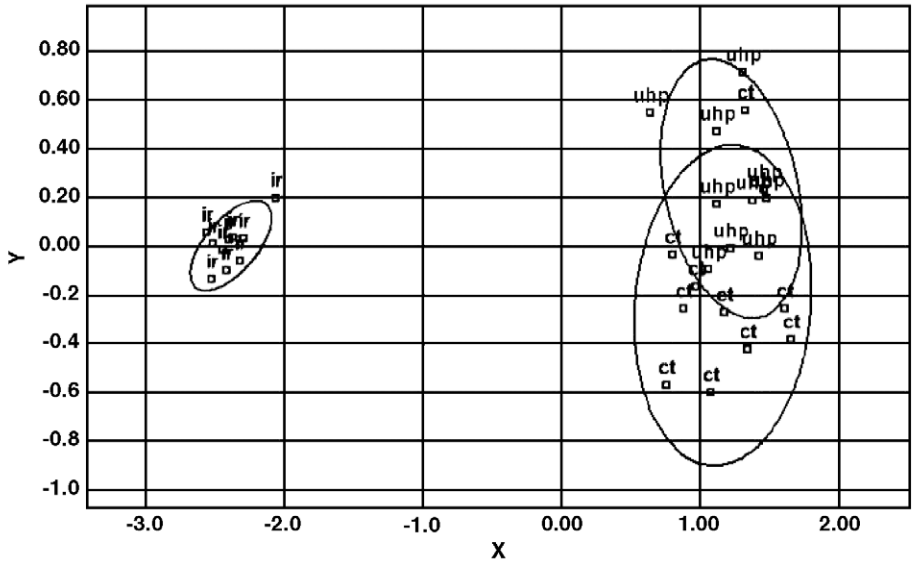

Fig. 8. The discriminant analysis score plot of the investigated egg white samples. The discriminant functions were calculated using the first 8 principal components of their 2nd derivative NIR spectra. ct: control, ir: 3 kGy, UHP: 400 MPa.

validated results are given for correct classification PQS shows the best overall separation, CDA and PCA+CDA are equal in separability, while PCA is the poorest. Table 3 (top) introduces the confusion (classification) matrix summarising the electronic nose classification results. It shows 100% correct classification of the UHP treated and irradiated egg white sample sets obtained by using Metrika's generalised SRT algorithm.

The same chain of ideas were followed during the comparative evaluation of our NIR investigations. Fig. 5 represents the PQS quality points of the investigated sample sets derived from the 2nd derivative spectra in the optimal wavelength range, while Figs. 6–8 show the PCA (Fig. 6) and CDA (Fig. 7) score plots of the same sample sets using the original 2nd derivative spectra and using the first 8 PCs for the calculation of the discriminant functions — the PCA+CDA (Fig. 8) score plot. Similar to Table 1 (top), Table 2 (top) shows the sensitivity values among the different sample sets using the applied evaluation methods for comparison. The results for separation using the NIR spectra are similar to the results in Table 1. The PCA+CDA method gives the best separation between control and irradiated samples, but it is poor in separating control and pressurised samples . CDA method is good in separating control and pressurised samples. The PQS method gives the best overall separation even using the NIR spectra of the samples according to the bottom of Table 2 in which full cross validated results are given. The percentage of the correctly classified samples decreased using PCA+CDA combination. Table 3 (bottom) representing the SRT confusion matrix shows that 100% of the samples are correctly classified using SRT method.

4. Conclusion

Based on the presented results it can be concluded that the irradiation (3 kGy) causes more drastic changes in the volatile compounds and in the NIR properties than the UHP treatment (400 MPa, 15 min, 4 °C). These confirm previous report [7] namely gamma irradiation led to significant changes in physical characteristic (conformational changes of egg components, e.g. aggregation or breakdown of proteins) and flavour of egg white. Despite this it was shown that important functional properties of egg white (e.g. whippability, foam stability) were not adversely affected by irradiation.

The non-linear SRT method provided 100% results in the evaluation of both electronic nose and NIR instrumentation.

Table 2
The numerical comparison of the applied methods for NIR spectra using the PQS sensitivity values (top) and the percent of correct classification in CDA cross validation (bottom)

	PQS	PCA	CDA	PCA+CDA
Control–irradiated	2.47	0.94	2.98	5.93
Control–UHP	1.58	0.27	1.58	0.61
Irradiated–UHP	2.08	1.25	2.78	7.05

	PQS	PCA	CDA	PCA+CDA
Originally grouped cases	100%	83.3%	100%	86.7%
Cross validated cases	87.8%	80.0%	66.7%	50%

Table 3
The SRT confusion (classification) matrix summarising the classification results of the differently treated egg white samples measured by electronic nose (top) and by NIR spectrometer (bottom) using Metrika's SRT algorithm

Egg white	Control	3 kGy	400 MPa
Control	100%	0%	0%
3 kGy	0%	100%	0%
400 MPa	0%	0%	100%

Egg white	Control	3 kGy	400 MPa
Control	100%	0%	0%
3 kGy	0%	100%	0%
400 MPa	0%	0%	100%

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