CALORIMETRIC STUDY OF CHANGES INDUCED BY PRESERVATIVES IN LIQUID EGG PRODUCTS

Csaba Németh, László Friedrich, József Surányi, Csaba Balla
Department of Refrigeration and Livestock Products Technology, Faculty of Food Science, Corvinus University of Budapest, 1118 Budapest, Ménesi út 43-45, Hungary
e-mail: csaba.nemeth@uni-corvinus.hu

ABSTRACT

In our tests we aimed to study the effects of additives normally used in liquid egg products on the calorimetric properties of liquid egg samples. Raw liquid egg white (pH=8.9±0.1), liquid egg yolk (pH=6.5±0.2) and whole liquid egg (pH=7.1±0.2) broken up in industrial conditions were used in our measurements. Sodium benzoate and potassium sorbate were added to the samples to achieve solutions of 0.1, 0.3 and 0.5 g/L concentrations. We used citric acid to prepare liquid egg products with various pH value (5.5, 5.0 and 4.5). The calorimetric tests were performed with MicroDSC III device. Liquid egg samples were heated up from 20°C to 95°C generally with a heating rate of 1.5°C/min. Sample weights were 500 mg±0.1 mg and water was used as reference solution. Evaluation was performed by Seftsoft2000 software, a component of the device.
Our measurements have shown significant changes in calorimetric parameters of liquid egg samples by decreasing the pH value to 5.0. In addition to the reduced enthalpy due to acidification of samples, a decrease in denaturation temperature was also observed in the egg white. While the native egg white started to precipitate at 60°C, at pH 5.0 the denaturation was already started at 54.5°C. When sodium benzoate and potassium sorbate were added to liquid egg products a significant change of the tested calorimetric values was only found in the liquid egg samples containing the preservative at the concentration of 0.5 g/L.

1. INTRODUCTION

Many additives are used in the food industry. Some of them are added to the food for increased food safety to improve the efficiency of anti-microbial treatments or to extend shelf-life [Ogihova *et al.*, 2009; Rangan *et al.*, 2009; Lee *et al.*, 2009]. Liquid egg manufacturers mainly use additives to extend shelf-life of these products. This is partly because customers using large quantities of liquid egg products prefer ready-for-use liquid egg products for the technology compared to powdered egg products, applicability and protein composition of which might be impaired [Landfeld *et al.*, 2008] but liquid egg products spoil rapidly even when stored in refrigerator.
When using additives, one should consider that calorimetric properties as well as heat sensitivity of liquid egg products (liquid whole egg, liquid egg white, and liquid egg yolk) may change [Torregiani *et al.*, 2009; Carmona *et al.*, 2007; Ichikawa *et al.*, 2007; Mizutani *et al.*, 2006]. This can be a problem mainly in case of preservation technologies where the preservatives are added to the liquid egg prior to heat treatment. Such procedures include treatment of liquid eggs in packaging for avoidance of the chance of possible post-infection during the packaging process.
From egg components, proteins are the most heat sensitive; proteins are found in high percentage in the egg white [HammershØj *et al.*, 2007; Rossi *et al.*, 1992; Gossett *et al.*, 1984]. Egg white is a protein system comprising ovomucin fibres incorporated into an aqueous solution containing numerous globular proteins. The most important representatives of these proteins – due to their amount – include ovalbumin, conalbumin (ovotransferrin), ovomucoid, ovomucin, lysozyme and globulins [Chang *et al.*, 1977].

Composition of egg yolk is more complex; it can be best described as a complex system in which diverse particles are suspended in a protein solution (livetin) [Nielsen, 1998].
DSC method has already been used several times to study thermal denaturation of egg white and its fractions [Ferreira *et al.*, 1997; Donovan *et al.*, 1976; Donovan *et al.*, 1975]. Particularly, it helps measuring the enthalpy of denaturation (ΔH_d) in case of egg white and some of its components, and thereby it is able to provide quantitative information. Furthermore, denaturation temperature of various proteins can also be measured with this method [Andrassy *et al.*, 2006; Zhang *et al.*, 2004; Mohácsi-Farkas *et al.*, 1999].

In this study we investigated the calorimetric effects of potassium sorbate and sodium benzoate used in egg processing and confectionery industry [Gliemmo *et al.*, 2004] and pH-reduction with citric acid in raw liquid egg products homogenized in the routine industrial manner.

2. MATERIALS AND METHODS

Materials

Raw liquid egg white (pH=8.9±0.1), egg yolk (pH=6.5±0.2) and whole egg broken (pH=7.1±0.2) under industrial conditions and homogenized in a piston-gap homogenizer at 100 bars were used in our tests. Sodium benzoate and potassium sorbate were added to the samples to achieve solutions of 0.1, 0.3, and 0.5 g/L concentrations. We used citric acid to prepare liquid egg products with various pH (5.5, 5.0, and 4.5).

CONSORT C831 model liquid pH-meter was used to control pH.

Differential Scanning Calorimeter (DSC)

Tests have been performed by using Setaram MicroDSC III device. Liquid egg samples were heated up from 20°C to 95°C at a heating rate of 1.5°C/minute.
The measured mass of liquid egg samples was 500mg ±0.1mg and water was used as reference. In some cases a second test cycle was also performed but no reversible phenomenon was observed.
Evaluation was performed by Seftsoft2000 program, a component of the device.

3. RESULTS AND EVALUATION

Analysis of homogenized egg products without preservative. First tests were carried out with raw liquid eggs without additives. Endothermic phenomena observed in the liquid egg white typically correspond to protein denaturation. However, from the 4 main components of egg white (conalbumin, lysozyme, ovalbumin, and globulins) fusion of peaks of lysozyme and conalbumin could be observed as a result of homogenization. Onset of denaturation was observed at 60°C while the peak (maximum) denaturation temperature was around 63.5°C
The second peak (onset of denaturation at 70 °C) is typical to denaturation of ovalbumin and globulins. Ratio of ovalbumin in liquid egg white is significantly higher (54% of total protein) compared to that of globulins (8% of total protein); therefore, peak parameters are determined by the calorimetric properties of ovalbumin. Peak denaturation temperature of these proteins was around 77°C.
Liquid egg yolk contains large amounts of lipoproteins. These cannot be separated well by calorimetric methods compared to the proteins of liquid egg white even in the non-

homogenized sample. Therefore we analyzed one peak for each sample i.e. total lipoproteins.
According to industrial experiences egg yolk is much less sensitive to heat than liquid egg white. Our tests have shown that peak denaturation temperature of these proteins was around 78 °C.
The calorimetric properties of liquid whole egg are influenced by the heat sensitive components of liquid egg white and egg yolk. The peak denaturation temperatures of ovalbumin in the homogenized liquid egg white and liquid egg yolk lipoproteins was similar (77-78°C), and the thermogram of liquid whole egg is predominantly influenced by these two fractions. In the tests, we only investigated changes affecting significant technological applicability; therefore, in case of liquid whole egg we investigated only that peak.

Analysis of homogenized egg products with preservative. Baseline and peak denaturation point as well as enthalpy of various native liquid egg white fractions have changed by pH-reduction with citric acid.
When pH of liquid egg white was reduced to 4.5 baseline denaturation point of the conalbumin-containing fraction reduced from 60°C to 45°C while denaturation enthalpy reduced by two-third (from 0.273 J/g to 0.092 J/g).
Denaturation temperatures of the ovalbumin-containing fraction considerably shifted. Reduction of pH of native liquid egg white (pH 8,0) to 4.5 reduced the baseline denaturation temperature from 72 °C to 64°C and the peak denaturation temperature from 77°C to around 70°C. Nevertheless, denaturation enthalpy decreased only slightly by pH reduction (from 1.075 J/g to 0.965 J/g at pH 4.5).
In case of pH reduction of native liquid egg yolk (pH 7,0) with citric acid, decrease in denaturation temperatures and denaturation enthalpy were measured.
For liquid egg yolk the baseline denaturation temperature has decreased to 64.5°C even at pH 5.5 compared to 69°C measured in native liquid egg yolk, but subsequently it did not change significantly. Contrarily, constant reduction in peak denaturation point has been found. While in native liquid egg white this value was around 78°C at pH 5.5, and reduced to around 74°C at pH 5.0, and 71°C at pH 4.5.
Changes in denaturation temperatures and denaturation enthalpy have been observed also in tests with liquid whole egg at various pH values. Baseline denaturation temperature has reduced from 70.5°C in the native liquid whole egg (pH 7,6) to below 65°C at pH 4.5 while peak denaturation point only slightly changed (measured range 75-78°C).
Denaturation enthalpy decreased from 1.738 J/g to 0.975 J/g by pH reduction to 4.5. The most significant change in enthalpy was found with pH reduction from 5.5 to 5.0 resulting in enthalpy change from 1.704 g/J to 1.300 g/J.
Assessment of calorimetric diagrams of native liquid eggs has shown results similar to literature data (Andrássy et al., 2006; Ferreira et al., 1997). From the egg proteins characteristics of conalbumin were changed the most significantly. Cunningham and Lineweaver (1965) have also drawn this conclusion when they established that 40% of conalbumin is precipitated with heat treatment at 57°C for 10 minutes in phosphate-bicarbonate buffer at pH 6. However, when conalbumin solution was adjusted to pH 9 and heated under the same conditions, the protein did not precipitate (Cunningham & Lineweaver, 1965).
When sodium benzoate and potassium sorbate were added to liquid egg no significant changes have been observed in calorimetric values compared to pH reduction; therefore, we only show DSC curves of liquid egg white samples that are more significant regarding the change in heat sensitivity.

No significant differences were measured in denaturation temperature for liquid egg white and egg yolk with preservative concentrations of 0.1 and 0.3 g/L. The course of the curve changed and it had two peaks meanwhile the position of denaturation peak was almost unchanged and the change in the area determining denaturation enthalpy was not significant either.

For liquid egg samples, the tests have shown smaller differences in denaturation temperature and enthalpy change of liquid egg products with added potassium sorbate and sodium benzoate compared to those with reduced pH, but we observed significant difference in liquid egg samples with 0.5 g/L sodium benzoate content. Example the baseline denaturation temperature of whole egg changed from 70.5° to 66.5 °C and peak denaturation temperature from around 78 °C to 72 °C. Potassium sorbate in concentration of 0.5 g/L. has changed denaturation temperature of liquid whole egg and liquid egg yolk by more than 2 °C compared to baseline.

Table 1: Changes in calorimetric properties of liquid egg product by pH reduction

pH of liquid egg products		**Liquid egg white**		**Liquid egg yolk**	**Liquid whole egg**
		Peak 1	**Peak 2**		
Native liquid egg	T(baseline)	60,34	71,93	69,14	70,49
	T(max)	63,52	77,06	78,35	77,91
	ΔH_d	0,2727	1,0750	1,7248	1,7384
pH 5,5	T(baseline)	60,00	70,58	64,66	68,01
	T(max)	62,47	76,46	77,75	77,94
	ΔH_d	0,1505	1,0598	1,4466	1.7040
pH 5,0	T(baseline)	54,47	67,77	64,42	67,04
	T(max)	57,31	73,73	73,90	75,13
	ΔH_d	0,1368	1,1155	1,1019	1,2229
pH 4,5	T(baseline)	45,12	63,72	64,25	64,78
	T(max)	48,99	70,28	70,79	76,76
	ΔH_d	0,0923	0,9647	0,4403	0,9753

T(baseline) *– baseline denaturation temperature [°C]*
T(baseline) *– peak denaturation temperature [°C]*
ΔH_d *- change in denaturation enthalpy [J/g]*

4. CONCLUSIONS

Our tests have demonstrated that preservatives used in acceptable concentrations change the protein structure and thereby calorimetric properties of liquid egg products i.e. baseline denaturation temperature. The measurements have shown significant changes in calorimetric parameters of liquid egg samples by decreasing the pH value to 5.0. In addition to the reduced enthalpy due to acidification of samples, a decrease in denaturation temperature was also observed in the egg white. While the native egg white started to precipitate at 60 °C, at pH 5.0 the denaturation was already started at 54.5 °C. When sodium benzoate and potassium sorbate were added to liquid egg products a significant change of the tested calorimetric values was only found in the liquid egg samples containing the preservative at concentrations of 0.5 g/L. Addition of preservatives to the native liquid egg prior to treatment should be considered in technologies.

In conclusion, the calorimetric test method has proved to be useful in the measurement of the effect of preservatives added to liquid whole egg as well as to liquid egg yolk and liquid egg white.

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