

## EFFECT OF NATURAL PRESERVATIVES ON THE SHELF LIFE AND CALORIMETRIC PROPERTIES OF SALTED, LIQUID WHOLE EGG

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### Abstract

Eggs are used for a wide variety of purposes in the food industry. Mostly ready for use egg products are used in the technology, for example, liquid whole egg. However, this product has limited shelf life (10 - 11 days) and there is a market need for products with longer shelf life.

In our study, we worked on the prolongation of pasteurized salted liquid whole egg's shelf life. We used different plant extracts and allyl isothiocyanate because there is an emerging trend of using natural preservatives. Among the used plant extracts were lavender, rosemary, ginger and sage extracts. First, we determined the appropriate concentrations of plant extracts with sensory tests. We prepared solutions in 10 different concentrations of the extracts in the range of  $10^2$  -  $10^4$  mg/L. An expert panel identified the maximal concentration of each extract based on the samples sensory properties. After that, a 4-week long storage experiment was performed. We defined total plate count and the number of *Enterobacteriaceae* of the samples with plate counting method every week. Besides that, we examined the calorimetric properties of samples with Differential Scanning Calorimetry. Statistical analysis was performed by IBM SPSS Statistics 22.0 software.

Samples were considered satisfactory under Hungarian regulations if their total number of bacteria did not reach  $10^5$  CFU/g and the number of *Enterobacteriaceae* was less than  $10^2$  CFU/g. Results of the microbiological analysis have shown, that allyl isothiocyanate preserves samples more than 4 weeks. Among the plant extracts, the use of ginger proved to be the best preservative, with a minimum shelf life of 21 days. At the

applied concentration the added natural materials did not significantly influence the calorimetric properties of the samples.

It was found that by adding different natural preservatives, the consumption time can be extended without affecting the calorimetric properties. In the next of our study, we investigate consumer preference and economic aspects.

**Key words:** Liquid whole egg, Shelf life, Calorimetric properties, Microbiological status, Plant extracts, Allyl isothiocyanate.

### 1. Introduction

Eggs are highly nutritive complete materials, which are used for a wide variety of purposes in the food industry. This is due to the fact that it is a balanced nutrient source. They are rich in proteins of high biological value, as well as polyunsaturated fatty acids, different trace elements or vitamins (Ju *et al.*, [1]). In recent years, food manufacturers substitute shell eggs with broken and pasteurized liquid egg products as liquid whole egg (LWE), or liquid white and liquid yolk since these products represent lower microbiological risk and their handling is easier (Németh *et al.*, [2]).

Even though fresh eggs are considered as sterile, some microorganisms can penetrate the shell (Uysal *et al.*, [3]). When the hen is laying eggs, the shell becomes contaminated with microorganisms of faeces and from the nest. During the production of liquid egg products, broken eggs provide an excellent medium for

microorganisms of the shell and environment (Nemeth *et al.*, [2]). The European Union does not set compulsory technological parameters for pasteurisation, but maximum levels for the microbiological status of the finished product are specified. The finished product must be free from *Salmonella* spp. (0 colony forming units (CFU)/25 g), and may not contain more than 100 CFU/g bacteria belonging to the *Enterobacteriaceae* family [4]. In addition, the Hungarian regulation also requires the maximum number of microbes, which is  $10^5$  CFU/g [5]. Conventional liquid egg pasteurization technologies (heat treatment at 60 - 65 °C for 5 - 10 min.) result products with a limited shelf life (10 - 11 days in case of LWE), because some of the surviving microorganisms can multiply under refrigerated conditions (Delves-Broughton *et al.*, [6]).

Unfortunately, components of LWE are sensitive to high temperatures, they cause the coagulation of proteins and quality deterioration e.g. loss of foaming, emulsifying and gelling capacities (Dawson and Martinez-Dawson, [7]). Longer shelf-life can be achieved by combined preservation methods based on Leistner's hurdle technology. According to the hurdle technology, an increase of the quality and improvement of nutritional value and sensory properties of foods can be achieved with the simultaneous use of several minor destructive treatments (Leistner, [8]).

Synthetic food additives mainly sodium benzoate and potassium sorbate are commonly used as preservatives in LWE. Nowadays, there is an increasing trend towards the use of different natural preservative agents and subjects, which can substitute synthetic food additives and they are "environmentally friendly" (Burt, [9]). Natural antimicrobials can be grouped by their origin, there are natural preservatives of plant origin, animal origin, bacterial origin, and there have been reports of algae and mushrooms as natural preservatives (Gyawali and Ibrahim, [10]). Among naturally occurring substances researchers and consumers have a special interest in plant-derived natural antimicrobials, essential oils (EOs), their individual components (ICs) and plant extracts (Burt, [9]). Plant EOs and extracts are used since ancient times for flavouring of food and as preservatives. However, the mechanism of action is not completely understood (Tajkarimi *et al.*, [11]). The antimicrobial activity of EOs, ICs and extracts have been tested by many researchers with *in vitro* tests and experiments have been carried out in food systems, as well. The general conclusion can be deduced, that a greater concentration of plant-derived preservatives is needed to reach the same effect in foods as under *in vitro* circumstances (Burt, [9]). In LWE, authors focused on different non-thermal preservation technologies, such as: pulsed electric field, high hydrostatic pressure, UV light, and ultrasound waves (Espina *et al.*, [12]). In recent years experiments have been carried out with

natural preservatives, the ICs carvacrol and cinnamaldehyde have been combined with heat treatment, ionising radiation and ultrasound technology (Alvarez *et al.*, [13], Valverde *et al.*, [14]) Espina *et al.* [12] tested three ICs: (+)-limonene, citral and carvacrol, and besides that three EOs: lemon EO, mandarin EO and rosemary EO, combined with PEF and heat treatments. Allyl isothiocyanate (AITC) has been tested as a substance of active packaging (Jin and Gutler, [15]).

When using different preservation technologies, calorimetric properties and heat sensitivity of LWE may change (Torregiani *et al.*, [16], Németh *et al.*, [17]). The most heat sensitive components of the egg are proteins, most of which are in the egg white (Hammersh *et al.*, [18]). The most important proteins include: ovalbumin, conalbumin, ovomucoid, lysozyme and ovomucin with different thermal sensitivity (Chang *et al.*, [19]). Differential Scanning Calorimetry (DSC) is able to measure the enthalpy of denaturation ( $\Delta H_d$ ) and the denaturation temperature of protein fractions can be determined (Andrássy *et al.*, [20]).

There is a market demand for the development of LWE products with a longer shelf-life. In this study, we aimed to extend the shelf-life of salted LWE with the addition of natural preservatives, which product is mainly produced for catering units and it is recommended for the production of: omelette, scrambled eggs, breaded products, pasta, mayonnaise, pancakes, soups, and sauces. Therefore we selected a solid vegetable extract (rosemary extract), three alcoholic extracts (ginger extract, sage extract and lavender extract) and an IC (AITC) to examine their effects on the sensory properties, shelf-life and calorimetric properties.

## 2. Materials and Methods

### 2.1 Materials

Freshly produced salted pasteurized LWE was used as material in our tests. The product has a 10-day shelf-life due to preservation procedures. Solid rosemary extract and three alcoholic extracts (ginger extract, sage extract and lavender extract) were added to the samples to achieve solutions of: 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, and 10 g/L concentrations. AITC (> 95% purity, Sigma Aldrich, USA) was added to the samples to achieve solutions of: 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 ppm concentrations.

### 2.2 Determination of the maximum concentration of preservative substances

First, we determined the appropriate concentrations of each substance with sensory tests. Scrambled eggs were made from the samples with the concentrations described in 2.1. They were cooked under identical

conditions without adding oil for 45 sec. without stirring, after that for 30 sec. with stirring. An expert panel sought to find out whether the individual samples were marketable based on their sensory characteristics (odour, colour, taste, texture and baking technology) or not. At the same time, 10 freshly made scrambled eggs were evaluated.

### 2.3 Storage experiment

Immediately after processing, we added to 100 mL of LWE the selected extracts and AITC to achieve the concentrations defined by the expert panel. We worked under aseptically circumstances and samples were measured in sterile flasks. A 28-day storage experiment was carried out. Samples were stored aerobically at 4 °C. Freshly produced LWE was stored under identical conditions as the control. Two samples containing AITC and extract as well as control were taken out aseptically and their microbiological quality was tested every week. In addition, the pH value (Testo 206) and the sensory changes (off-odour, coagulation) of all the samples were followed during the experiment.

### 2.4 Microbiological analysis

Total viable count and the number of *Enterobacteriaceae* was determined every week by pour plate method to investigate the antimicrobial effect of the added substances. Tenfold dilution series of the control and the samples were made in peptone water (Biokar, France). Total viable count was identified with Nutrient agar (Biokar, France) after 48 hours of incubation at 37 °C. The number of *Enterobacteriaceae* was determined with Gelose VRBG agar (Biokar, France) after an incubation for 24 hours at 30 °C. After incubation, the CFU were enumerated and expressed as  $\log_{10}$  CFU/g. The detection limit was 10 CFU/g.

### 2.5 Differential Scanning Calorimetry

Calorimetric properties of the samples with added AITC and extracts and of the control were determined with DSC before storage experiment. Calorimetric tests were performed with MicroDSC III instrument. The measured mass of the samples was in each case 7780.1 mg. Liquid egg samples were heated up from 20 °C to 95 °C with a heating rate of 1.5 °C/min, the cooling rate was 3 °C/min. Distilled water was used as reference solution and evaluation was performed by Callisto Processing software. For the heat flow curve was set a straight baseline. After that, we determined the denaturation temperature (Peak Maximum, °C) and the area under the curve, which represents the enthalpy of denaturation (Heat, J/g).

### 2.6 Statistical evaluation

Denaturation temperature and denaturing enthalpy values of samples containing different plant-derived compounds were compared to the control sample with t-test for independent samples. The aim was to determine whether the used substances change the thermal denaturation properties of the proteins. Statistical analysis was performed by IMB SPSS Statistics 22.0 software. Normality test and Levene's test were performed to test normality and equality and significance was based on  $p < 0.05$ .

## 3. Results and Discussion

### 3.1 Specified concentration of preservative substances

First, we determined the concentrations of different extracts and AITC with the help of an expert panel. The concentrations shown in Table 1 were determined, which do not yet significantly affect the organoleptic properties of the scrambled eggs made of the samples. It can be seen that among the plant extracts lavender (1 g/L) and ginger extract (2 g/L) can be added to LWE at lower concentrations. Lavender extract in greater concentration results a floral off-odour, ginger makes LWE bitter if used at higher concentrations. In contrast, a higher concentration can be applied of sage (4 g/L) and rosemary extracts (7 g/L), because they are better suited for the egg scavenging's sensory properties. Sage in greater concentration, than the applied 4 g/L makes egg scavenging greenish and it has a spicy taste. AITC can be added in the concentration of 0.5 ppm to the LWE, a strong mustard off odour appears in case of higher concentrations.

**Table 1. To LWE added maximum natural preservative concentrations defined by the expert panel**

| Extract name     | Concentration (g/L) |
|------------------|---------------------|
| Sage extract     | 4                   |
| Rosemary extract | 7                   |
| Ginger extract   | 2                   |
| Lavender extract | 1                   |
| IC name          | Concentration (ppm) |
| AITC             | 0.5                 |

### 3.2 Results of the microbiological analysis

A 28-day storage experiment was performed to examine whether at the established concentrations there is an antimicrobial effect of the applied natural substances on the salted LWE. We determined total viable count and the number of *Enterobacteriaceae* weekly by pour

**Table 2. Evolution of total viable cell count and *Enterobacteriaceae* during the storing experiment**

| Mode of preservation             | Storage time (days) |        |                     |        |                     |        |                     |        |
|----------------------------------|---------------------|--------|---------------------|--------|---------------------|--------|---------------------|--------|
|                                  | 7                   |        | 14                  |        | 21                  |        | 28                  |        |
|                                  | Log <sub>10</sub> * | S.D.** | Log <sub>10</sub> * | S.D.** | Log <sub>10</sub> * | S.D.** | Log <sub>10</sub> * | S.D.** |
| <b>Total viable count</b>        |                     |        |                     |        |                     |        |                     |        |
| Control                          | 2,99                | 0.24   | 4,26                | 0.23   | <b>5,08</b>         | 0.06   | <b>5,92</b>         | 0.23   |
| Sage extract                     | 2,45                | 0.19   | 3,76                | 0.17   | 4,91                | 0.06   | <b>5,87</b>         | 0.32   |
| Rosemary extract                 | 2,76                | 0.09   | 4,20                | 0.09   | <b>5,53</b>         | 0.12   | <b>6,38</b>         | 0.25   |
| Ginger extract                   | 1,96                | 0.27   | 2,75                | 0.21   | 3,89                | 0.24   | <b>5,04</b>         | 0.18   |
| Lavender extract                 | 3,57                | 0.12   | 4,08                | 0.16   | <b>5,11</b>         | 0.12   | <b>6,32</b>         | 0.12   |
| AITC                             | 1,72                | 0.07   | 2,00                | 0.18   | 2,46                | 0.15   | 2,92                | 0.14   |
| <b><i>Enterobacteriaceae</i></b> |                     |        |                     |        |                     |        |                     |        |
| Control                          | <1                  | -      | <b>2,14</b>         | 0.12   | <b>2,71</b>         | 0.11   | <b>3,32</b>         | 0.06   |
| Sage extract                     | <1                  | -      | 1,62                | 0.11   | <b>2,20</b>         | 0.14   | <b>2,86</b>         | 0.18   |
| Rosemary extract                 | <1                  | -      | <b>2,17</b>         | 0.09   | <b>2,68</b>         | 0.08   | <b>3,92</b>         | 0.10   |
| Ginger extract                   | <1                  | -      | <1                  | -      | 1,51                | 0.07   | <b>2,15</b>         | 0.08   |
| Lavender extract                 | <1                  | -      | <b>2,34</b>         | 0.07   | <b>2,79</b>         | 0.12   | <b>3,36</b>         | 0.15   |
| AITC                             | <1                  | -      | <1                  | -      | <1                  | -      | <1                  | -      |

\* Average values for Log<sub>10</sub> CFU.

\*\* Standard deviation of Log<sub>10</sub>.

- Numbers with red bold characters indicate, that the value is over the maximal allowed values (100 CFU/g of *Enterobacteriaceae*, 10<sup>5</sup> CFU / g of total viable cell count).

plate method. Samples were considered satisfactory under Hungarian regulations if their total viable count did not reach 10<sup>5</sup> CFU/g and the number of *Enterobacteriaceae* was less than 10<sup>2</sup> CFU/g (Table 2).

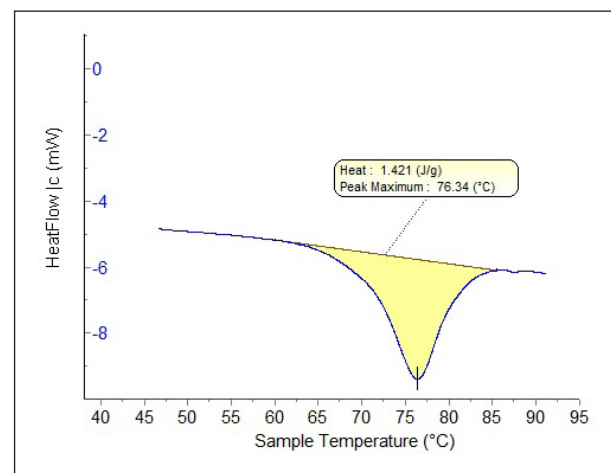
According to the Table 2, control sample no longer meets the requirements on day 14. The samples containing lavender and rosemary extract also exceed the permitted values. From this, we can conclude that at the applied concentrations they are not suitable for the extension of salted LWE's shelf life. With the addition of sage extract, we can improve the microbial state of our sample, the shelf-life is longer about a week in this case, as of the control. Ginger extract resulted the best microbiological state, the sample does not even exceed the specified levels even on day 21, and the shelf life is between 21 and 28 days. If we examine the results of the sample to which AITC was added, we can see that its microbiological status remained stable until the end of the storage experiment. In this samples, *no Enterobacteriaceae* can be detected during the 28-day long storage. However, microbiological studies were performed with the simultaneous observation of sensory changes (e.g. off-odour, coagulation, colour changes). We found, that samples with added AITC had a strong mustardy off-odour on day 7.

### 3.3 Calorimetric properties

The aim of the calorimetric study was to examine whether the added substances change the calorimetric properties of salted LWE. Therefore we examined

the shape of the heat flow curves first. The egg white contains 4 main components. These are the conalbumin, lysozyme, ovalbumin and globulin fractions (Németh *et al.*, [17]). But DSC measurements showed only one peak in each case (see Figure 1). The reason of this is, that the material we worked with is a processed product, conalbumin protein fraction denaturated and fragmented during the processing. In addition to this, the fusion of peaks could be observed.

We set a straight baseline to the heat flow curves of the samples and we determined the denaturation temperature (Peak Maximum, °C) and the enthalpy of denaturation (Heat, J/g) - Table 3.



**Figure 1. Example for heat flow curve (salted LWE with sage extract)**

**Table 3. Denaturation temperature and enthalpy of LWE samples**

| Mode of preservation | Denaturation temperature [°C] |      |       | Enthalpy of denaturation [J/g] |       |       |
|----------------------|-------------------------------|------|-------|--------------------------------|-------|-------|
|                      | Mean                          | S.D. | Sign* | Mean                           | S.D.  | Sign* |
| Control              | <b>77,41</b>                  | 0,04 | -     | <b>1,774</b>                   | 0,116 | -     |
| Sage extract         | <b>76,34</b>                  | 0,00 | x     | <b>1,504</b>                   | 0,117 | -     |
| Rosemary extract     | <b>76,56</b>                  | 0,03 | x     | <b>1,346</b>                   | 0,058 | -     |
| Ginger extract       | <b>77,44</b>                  | 0,06 | -     | <b>1,449</b>                   | 0,078 | -     |
| Lavender extract     | <b>76,77</b>                  | 0,15 | -     | <b>1,455</b>                   | 0,102 | -     |
| AITC                 | <b>77,40</b>                  | 0,05 | -     | <b>1,580</b>                   | 0,015 | -     |

\* Sign – significant difference based on the t-test for independent samples from the control sample.  
 x- There is significant difference between the sample and the control.

According to Table 3, no significant difference was observed between the denaturation enthalpy of the control sample and treated samples. If we observe denaturation temperature values, it can be seen that sage and rosemary extracts have resulted significant changes. This is probably due to the fact that the highest concentrations were used from these two extracts.

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### 4. Conclusions

- In this study, we worked on a market-based problem, on the extension of the shelf-life of salted LWE. We used different plant extracts and AITC because there is an increscent trend towards the use of different natural preservative agents. We found, that among the used extracts, ginger extract is capable to extend the shelf-life without changing the organoleptic and calorimetric properties of the scrambled egg made of the sample.

- In addition to this, AITC resulted microbiological stability of salted LWE during the 4-week long storage experiment.

- We found that samples with added AITC had a strong mustardy off-odour on day 7. It cannot be used at this concentration for the prolongation of salted LWEs shelf-life. Therefore, it would be useful to examine the effect of AITC at concentrations below 0.5 ppm on the shelf-life and organoleptic properties of the product.

- We consider it a useful research field, to use different natural preservative substances and agents. In our next study, we investigate consumer preference of different natural compounds in LWE and examine the economic aspects. In addition to this, we determine the accurate shelf-life of salted LWE, to which are added natural preservatives.

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