Baicalin, Added as the Only Preservative, Improves the Microbiological Quality of Homemade Mayonnaise

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Abstract: The purpose of a study was to determine if the baicalin, isolated from baical skullcap (Scutellaria baicalensis Georgi) root and added as the only preservative, would improve the shelf life of homemade mayonnaise. The experiment was performed on mayonnaise free from organic acids or preservatives but added the preparation of 95% baicalin (500 µg x g⁻¹). The material was kept at 10°C or 20°C with microbiological analyses performed on 0, 1, 3, 7 and 14th day of storage. Baicalin was revealed to affect positively the microbiological quality of the product. The microbiological counts in experimental mayonnaise were lower than in the controls during the entire period of storage. The antibacterial activity of baicalin depended on the incubation temperature. The shelf life of mayonnaise stored at 10°C was significantly prolonged comparing to product kept at 20°C. Concluding, the application of baicalin as the natural preservative of food seems highly promising.

Key words: Baicalin, bio-preservative, mayonnaise, shelf life

Introduction
Research on natural bactericidal or bacteriostatic compounds with possible application as biological preservatives is an important direction in consumer safety protection and food technology or microbiology. Baicalin, the flavonoid from the root of baical skullcap (Scutellaria baicalensis, Georgi), perennial plant from Lamiales family (Labiateae), seems to follow the criteria of biological preservative. Literature data, together with the experience of Far East medicine, indicate that the aforementioned compound have various therapeutic effects and might be applied in the adjunct treatment of many disorders (Martin and Dusek, 2002).

There also exist some data on the antibacterial activity of baicalin, but the results of documented experiments are not unequivocal. Kubo et al. (1981) determined the minimal inhibitory concentration (MIC) of baicalin against Staphylococcus aureus and Escherichia coli as higher than 250 µg x ml⁻¹. Ng et al. (1996) revealed that baicalin at concentration 128 µg x ml⁻¹ inhibited the growth of 11/16 strains of S. aureus and 2/6 of Pseudomonas aeruginosa. Conversely, Cushnie et al. (2003) failed to determine MIC against any of the strain studied (S. aureus, Escherichia coli and P. aeruginosa), using baicalin at maximal concentration of 100 µg x ml⁻¹. Surprisingly low MIC of baicalin against S. aureus, 64 µg x ml⁻¹, was in turn reported by Liu et al. (2000).

Although the application of baicalin in antibacterial therapy seems rather limited due to its relatively weak action, the substance might be considered as a biological preservative. Accordingly, the purpose of a study was to determine if the baicalin, isolated from baical skullcap root and added as the only preservative, would improve the shelf life of homemade mayonnaise.

Materials and Methods
The experiment was performed on mayonnaise (1500 g) made of hen egg yolks and sunflower oil, without organic acid or preservative addition. The yolks were homogenized (Thermomix TM 21, Vorwerk & Co. KG, Wuppertal, Germany) and gradually added the oil (ca 30 ml per one 15-17 g yolk). One third of mayonnaise was left as the controls, while the remaining 1000 g was added the preparation of 95%baicalin isolated from the root of baical skullcap (500 µg x g⁻¹, Wimex Ltd, Beijing, China).

The material was divided into 10 g or 25 g samples and stored at 10°C or 20°C (thermostatic chamber ST 700, Pol-Eko Aparatura S.J., Wodzislaw Słaski, Poland), until the visible signs of microbiological decay were visible. Microbiological analyses were performed on 0, 1, 3, 7 and 14th day of storage. The following microbiological counts were determined per 1 g of sample: total plate...

30
Bruzewicz et al.: Baicalin Improves the Microbiological Quality of Homemade Mayonnaise

Fig. 1: Total plate count in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g⁻¹ (●). Values with different letters are significantly different (P≤0.05). Error bars represent standard deviations.

Fig. 2: The number of psychrotrophic bacteria in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g⁻¹ (●). Values with different letters are significantly different (P≤0.05). Error bars represent standard deviations.

Fig. 3: The number of coliforms in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g⁻¹ (●). Values with different letters are significantly different (P≤0.05). Error bars represent standard deviations.

count (agar medium, 37°C, 24h, PN-EN ISO 4833:2004), psychrotrophic bacteria (agar medium, 22°C, 24h, PN-ISO 17410:2004), coliforms (Chromocult® Coliform Agar, Merck KGaA, Darmstadt, Germany, 37°C, 24h), coagulase-positive staphylococci (Baird-Parker Agar Base, Merck KGaA, Darmstadt, Germany, 37°C, 48h), yeasts and moulds (agar medium with yeast extract, glucose and chloramphenicol, 25°C, 24h, PN-ISO 7954:1999). Moreover, 25 g samples were examined for Salmonella spp. (BPLS Agar, Merck KGaA, Darmstadt, Germany, 37°C, 24h). Each microbiological determination was performed on 5 samples, whereas the entire experiment was repeated 7 times, which corresponded to the total number of 210 samples examined per every storage day.

Microbiological counts were transformed into logarithms and statistical calculations were carried out using Statistica 5, Version 97 (StatSoft® Polska Sp. z o.o., Krakow, Poland) package. The importance of the differences was established with the aid of Student’s test (P≤0.05).

Results

Storage at 10°C: Microbiological analyses were carried out by the 15th day of storage, when the superficial growth of moulds disabled further determinations. The storage-related changes in total plate count and in the number of psychrotrophic bacteria are presented in Fig. 1-2.

Coliforms were not detected in any sample of the baicalin-added mayonnaise during the entire period of storage whereas they were demonstrated in the controls on the days 7 and 14 (Fig. 3). Similarly, by the end of the experiment no yeasts and moulds were isolated from the baicalin-containing product. After 24 hours of the study saprophytic fungi were however demonstrated in the controls and their number increased gradually with further storage (Fig. 4). Salmonella spp. and coagulase-positive staphylococci were not demonstrated either in the baicalin-containing product or in the controls by the 14th day of storage at 10°C.

Storage at 20°C: Microbiological determinations were performed on 0, 1 and 3rd day of storage. The superficial growth of moulds, either on baicalin-containing mayonnaise or in the controls, was the reason to quit the further analyses.

The storage-related changes in total plate count and the number of psychrotrophic bacteria are presented in Fig. 5-6.

At the onset of experiment yeast and moulds were detected neither in the controls nor in the baicalin-added mayonnaise. The significant growth of the microorganism analyzed was however demonstrated after 24 hours of storage in both the variants of product.
The superficial growth of moulds, seen macroscopically after 5 or 15 days of storage at 10°C or 20°C, respectively, would suggest that the substance studied was ineffective against these microorganisms. The other authors however claimed on the efficiency of baikalin flavonoids against various pathogenic fungi (Yang et al., 1995, Biaaszczyn et al., 2000). Since in our study moulds were not isolated from the center of product by 3rd and 14th day of storage at 10°C and 20°C, respectively, their growth on the surface of experimental mayonnaise on days 5 and 15 reflects rather the time-related imbalance in baikalin distribution than the ineffectiveness of the substance studied itself.

Our study revealed that the antibacterial activity of baikalin depended on the temperature of product storage. The shelf life of mayonnaise stored at 10°C was significantly prolonged comparing to product kept at 20°C. Consequently, baikalin was proved to retain or even improve its antimicrobial properties at near refrigeration temperature. The aforementioned phenomenon is noticeable, since the short duration of the antibacterial effect of the compound studied, observed at ambient temperature, would be undoubtedly the main limitation for its use as a natural preservative of food.

Although the question was not verified by the present study, some literature data suggest that baikalin might be also applied as the natural preservative of products exposed to thermal treatment. The active compounds of 

Scutellaria root retain their antibacterial properties although they are usually extracted at 80-85°C, hence at near pasteurization temperatures (Tsao et al., 1982; Ng et al., 1996).

The question of toxicity definitely limits the spectrum of substances which might be used as natural preservatives. The amount of the baikalin added to mayonnaise in the present experiment was determined in course of the previous test strain studies (Bruzewicz, 2005). Although the detailed experiments on baikal skullcap flavonoids toxicity were not performed as far, the literature data indicates, that administered orally, these compounds do not exhibit any adverse effects (Havsteen, 2000).

Moreover, the results of numerous experiments, reviewed by Martin and Dušek (2002), suggest that baikalin and the other flavonoids of

Scutellaria baicalensis might exhibit several positive health effects when administered orally.

According to Chinese authors, the antibacterial activity of baikalein, the aglycon of baikalin, is even higher than of the latter. The concentration of baikalein in natural stock is however much lower (Tsao et al., 1982). Intestinal microflora of mammals and human is however known to degrade flavonoids ingested to their aglycones (Aherne and O'Brien, 2002). That effect was also observed for baikalin, which was transformed into baikalein by the intestinal bacteria (Kim et al., 1998; Zuo et al., 2002; Lin
Bruzewicz et al.: Baicalin Improves the Microbiological Quality of Homemade Mayonnaise


et al., 2003). Accordingly, it is very likely that baicalin, administered with food orally, would not lose but even improved its antimicrobial properties, consequently normalizing the intestinal microflora and preventing the alimentary intoxications.

Concluding, the application of baicalin as the natural preservative of food seems highly promising. The technological studies on the sensory properties of the baicalin-preserved products are however the next step towards the industrial application of substance studied.

References
