PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 5 (1): 30-33, 2006
ISSN 1680-5194

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 *Lamiaceae* family (*Labiatae*), 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 Dušek, 2002).

There also exist some data on the antibacterial activity of baicalin, but the results of documented experiments are not equivocal. 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 2/8 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 Slaski, 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

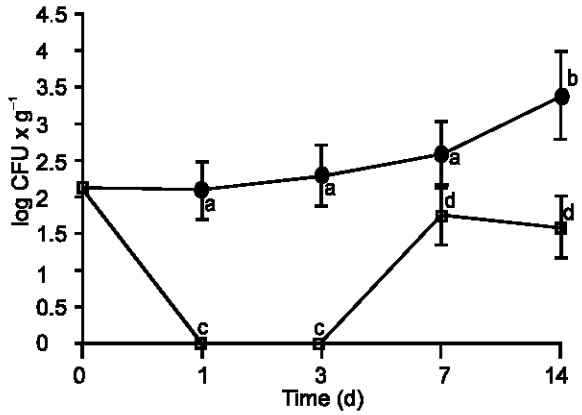

Fig. 1: Total plate count in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

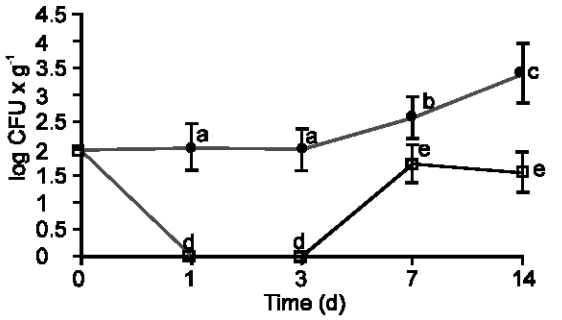

Fig. 2: The number of psychrotrophic bacteria in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

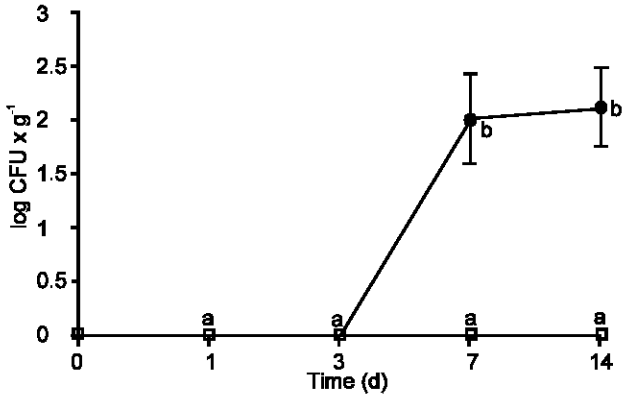

Fig. 3: The number of coliforms in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

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=.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.

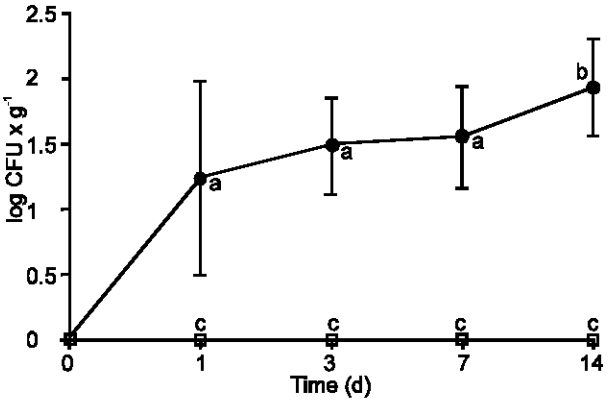

Fig. 4: The number of yeasts and moulds in homemade mayonnaise stored at 10°C. Control (●), baicalin 500 µg x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

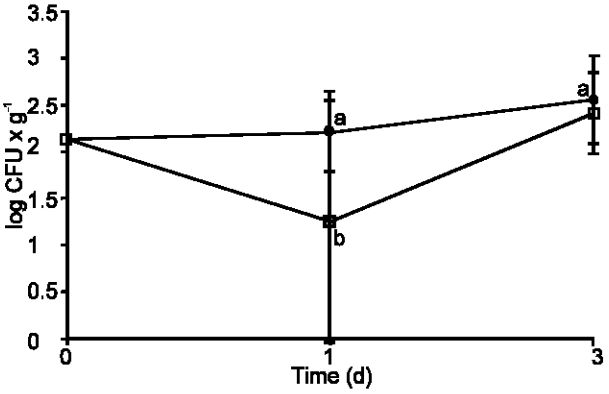

Fig. 5: Total plate count in homemade mayonnaise stored at 20°C. Control (●), baicalin 500 µg x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

The counts observed in the controls were still higher than in the baicalin-containing mayonnaise (Fig. 7).

Neither coliforms nor coagulase-positive staphylococci and *Salmonella* spp. were detected in experimental and control samples by the 3rd day of storage.

Discussion

According to our knowledge and on the basis of extensive search through the literature databases, the present experiment was the first one, which considered baicalin as a bio-preservative of food. Our study revealed that the flavonoid from the root of *Scutelllaria baicalensis*, used as the only preservative of homemade mayonnaise, positively affected the microbiological quality of the product. Accordingly, the microbiological counts in experimental mayonnaise were lower than in the controls during the entire period of storage.

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 baical skullcap flavonoids against various pathogenic fungi (Yang *et al.*, 1995, Blaszczyk *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 baicalin distribution than the ineffectiveness of the substance studied itself.

Our study revealed that the antibacterial activity of baicalin 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, baicalin 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 baicalin 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 baicalin added to mayonnaise in the present experiment was determined in course of the previous test strain studies (Bruzewicz, 2005). Although the detailed experiments on baical 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 baicalin and the other flavonoids of *Scutellaria baicalensis* might exhibit several positive health effects when administered orally.

According to Chinese authors, the antibacterial activity of baicalein, the aglycon of baicalin, is even higher than of the latter. The concentration of baicalein 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 baicalin, which was transformed into baicalein by the intestinal bacteria (Kim *et al.*, 1998; Zuo *et al.*, 2002; Lin

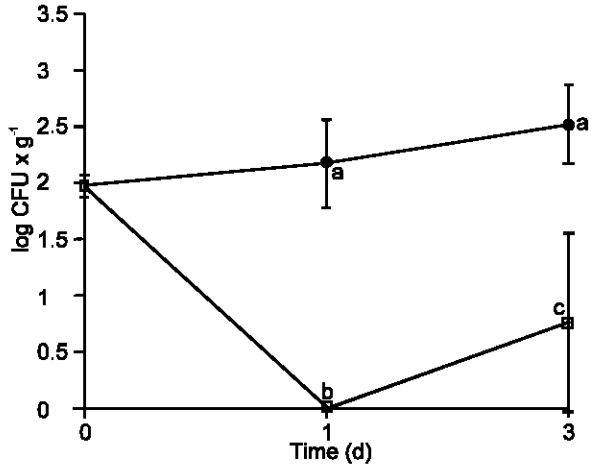

Fig. 6: The number of psychrotrophic bacteria in homemade mayonnaise stored at 20°C. Control (●), baicalin 500 µg x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

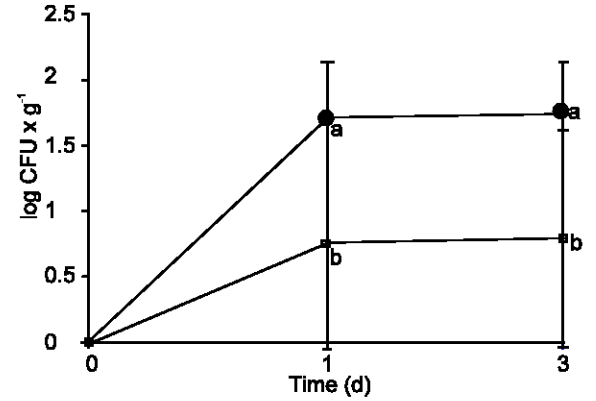

Fig. 7: The number of yeasts and moulds in homemade mayonnaise stored at 20°C. Control (●), baicalin 500 g x g^{-1} (□). Values with different letters are significantly different ($P \leq 0.05$). Error bars represent standard deviations

et al., 2003). Accordingly, it is very likely that baicalin, administered with food orally, would not lost 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.

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