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Controlling the Risk of *Bacillus* in Food Using Berries

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Abstract

Introduction: Increasing the shelf life of foods without the addition of synthetic additives is a demand from both producers and consumers. Spore-forming bacteria are a problem in the food industry. To reduce their impact, it is necessary to use complex technologies, as well as ingredients with antibacterial or antibiotic properties. The aim of this study was to develop initial symbiotic combinations between lactic acid bacteria and berries to control food quality. The relevant ability of lactic acid bacteria in the presence of berry additives to inhibit the growth of Bacillus strains that degrade bakery products and dairy products was investigated. The antibacterial effect of berries on the growth of *Bacillus mesentericus* was studied. **Methods:** In this study was used inhibition zone test, also called Kirby-Bauer Test. The growth rate of bacteria was based on the measurement of the optical density at 600 nm (OD600). The method of Thompson *et al.* has been used to research the development of ropiness disease in wheat bread samples. **Results:** The diameter of the *Bacillus pumilus* growth inhibition zones under the berries action was as follows: aronia −18.0 ± 0.6 mm; raspberry −16.0 ± 0.4 mm; strawberry −15.0 ± 0.5 mm. Lactic bacteria in the presence of berry additives showed a growth rate, measured by optical density (OD) at 600 from 0.073 to 0.651 (for aronia) from 0.071 to 0.609 (for raspberries), from 0.073 to 0.597 compared to the increase in environments without added fruit, which amounted to −0.050 to 0.410. In the yogurt with added fruit, a synergism was formed with *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp. Bulgaricus*, *Lactococcus lactis subsp Lactis biovar diacetilactis*. The influence of fat-soluble extracts of sea buckthorn, rose-hip, and hawthorn fruits on the development of ropiness disease when storing wheat bread was investigated. The general Pearson coefficient (microbial count and pH) for all fruit yogurt samples is 0.95066. **Conclusion:** The combined use of lactic acid bacteria and berries (aronia, raspberry and strawberry) had a synergistic effect on the risk posed by *Bacillus* bacteria. 1% of fat-soluble extract from the vegetable matter reduced the risk of ropiness disease in wheat bread. This is due to the cumulative effect of the berries chemical composition (antioxidants, organic acids, etc.), increased acidity, lowered pH, and water activity of the food environment, below the development values of *Bacillus*.

Keywords

Risk of *Bacillus*, Berries, Lactic Acid Bacteria, Fermentation, Synergism, Bakery and Dairy Products

1. Introduction

Preventing food spoilage without the addition of chemical food additives, while increasing functional properties of wheat-based bakery products, is an increasing demand by the consumers and a challenge for the food industry [1]. Functional food according to the European Commission Concerted Action on Functional Food Science in Europe (FuFoSE) and the International Life Sciences Institute (ILSI) is a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of a disease risk [2]. A food can be made functionally by increasing the concentration, adding, or improving the bioavailability of a particular component [3]. Certainly, the last decade, when consumer preferences have shifted to mildly processed food, new opportunities arise for spore-forming spoilage and pathogenic organisms [4].

Bakery products have a very short shelf life. Their quality depends on the time interval between baking and consumption [5]. Spoilage of bakery products is mainly due to the growth of moulds, the main species belonging to the genera *Aspergillus*, *Fusarium* and *Penicillium*, as well as to the roping of the bread, caused by *Bacillus sp.*, especially *Bacillus subtilis* and *Bacillus licheniformis* [6]. Statistics show that over the past decade, the number of small and medium-sized enterprises engaged in the production of bread and bakery products using non-traditional vegetable raw materials such as bran, seeds, grains, and legumes has increased. Such products are in demand among consumers, but the raw material is often a source of *Bacillus* bacteria, which requires the use of safe preservatives [7]. Considering the high microbiological contamination of raw materials (mainly flour) in the production of bread and bakery products, it is necessary to use complex technologies, as well as products with antimibacterial or antibiotic properties, to prevent the development of a ropiness disease and molding of bread [8].

Spore-forming bacteria can be a problem in the food industry, especially in the canning industry. Spores present in ingredients or present in the processing environment severely challenge the preservation process since their thermal resistance may be very high [9].

Cow's milk, which is one of the most complete foods [10], is at the same time a favorable breeding ground for microorganisms [11] [12]. The quality and stability of dairy products are largely determined by the initial bacterial contamination and the composition of the raw material microflora [11] [12] [13]. Optionally anaerobic sporulating microorganisms (*Bacillus subtillis*, *Bacillus mesentericus* (*Bacillus pumilus*), *Bacillus megatherium*, *Bacillus mycoides* and *Bacillus sereus*) have pronounced proteolytic properties, coagulate, and peptonize milk, cause hemolysis, release ammonia, hydrogen sulfide [14]. Research in recent years has established that these are significant microorganisms of damage to dairy products and food poisoning [15] [16]. Recent studies conducted by us have shown that the risk control posed by bacteria of genus *Bacillus* could be achieved by using berries in food manufacturing recipes. Fruits, berries medicinal plants have bactericidal and bacteriostatic properties [17]-[23]. Therefore, Pashenko *et al.* [24] developed a technology for producing rowan leaven, which included rowan powder, beet sugar molasses and kefir fungus. Leaven is a water-molasses extract fermented with kefir fungus. The symbiosis of lactic acid bacteria and kefir yeast cells has fungicidal and bactericidal activity due to the high content of sorbic acid, tannins, phytoncides and flavonoids.

To ensure microbiological quality and consumer safety, strict controls and hygienic conditions are observed by the dairy industry, as recommended by guidelines of good dairy farming [25] [26] and hygienic conditions at dairy plants [27].

The control of environmental parameters (temperature, pH, water activity, salinity, atmosphere, presence of additives) can help the control of *Bacillus cereus* proliferation in foods. The ability to produce spores makes *Bacillus cereus* capable to escape processing conditions carried out by the food industries to preserve products and to eliminate or reduce the bacterial number in the final product [28]. Heat treatments commonly used by the food industry require longer treatments to efficiently eliminate *Bacillus cereus* spores [29].

The objective of the study was to evaluate the antimicrobial effect of different berries on the development of *Bacillus* bacteria in bakery and dairy products. Also, it was evaluated the combined effects of pasteurization intensity (no heat treatment and 10 min at 70˚C, 80˚C and 90˚C), water activity (a_w) (0.960 - 0.990), pH (5.5 - 7.0) and storage temperature (7˚C and 10˚C) on the survival and outgrowth of psychrotolerant spores of *Bacillus cereus* FF119b and *Bacillus pumilus* FF128a [30].

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