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Effectiveness of a Natural Preservative Based on Grapefruit Seeds to Replace the Chemical Preservative SIN202 in Cheese Production

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ABSTRACT

Grapefruit seed extract is highly valued for its antibacterial and antifungal benefits, but unfortunately it is not available in the Algerian food industry or market in general. Our goal is to attempt the production of this extract as well as using two types of bought GSE, and then carry out an in-depth study to determine whether it could be used as a food preservative in the biggest nests of bacteria (Cheese). This article presents an experimental study aiming to replace the preservative E202 (or SIN202) aka Potassium sorbate in cheese production. We conducted laboratory tests to evaluate the effectiveness of grapefruit seeds as a natural preservative. The results showed remarkable antifungal effectiveness of GSE powder and GSE "Dioter" at a concentration of 2%, thus equalling the effectiveness of E202. The GSE Aroma-Zone enabled the inhibition of Staphylococcus Aureus, E.Coli and Candida Albicans at the lowest concentration of 0.5%, demonstrating its antibacterial and antifungal effectiveness, and surpassing the E202.

Keywords: Grapefruit Seed Extract (GSE), antibacterial, Potassium sorbate, cheese.

1 Introduction

The bacteria that can infect or contaminate cheese can vary depending on a number of factors. The most common are Escherichia Coli, Staphylococcus Aureus and Salmonella, which can produce toxins that causes food poisoning or serious infections. GSE is made by first converting dried grapefruit seeds into a powder which can be used as it is or can be turned into a liquid by adding glycerin, water or alcohol to it. Several experiments have been carried out on GSE over the years to prove its inhibitory efficacy on different strains of bacteria. Researches attribute this activity to the presence of Flavonoids such as Naringin and Limonoids, Quercetin, Kaempferol, Citric acid and other compounds [1]. According to the NATURVEDA laboratory, these compounds have demonstrated significant efficacy against a range of bacteria, including those mentioned above. In 1989, Ionescu J. et al. used a dietary supplement made from Grapefruit Seeds, called "ParaMicrocidin" (PM), against a diverse range of 194 bacterial and 93 fungal strains. [2]. Additionally a study carried out by Reagor et al. in 2002, evaluating the inhibitory activity of GSE on 67 different bacterial strains, showed that the GSE proved systematically antibacterial on all the strains tested [3]. In the food industry, a study by Choi et al. in 2014 analyzed the antibacterial effect of an GSE purchased in the USA with 30% glycerine and 2.19% Naringin, and another purchased in Brazil with 50% glycerine and 0.48% Naringin, on a traditional rice wine with an alcohol content of 6 to 7%, also containing vitamins, sugars, proteins and various organic and bioactive compounds. [4]. GSE has also been used for the preservation of foods such as vegetables [5][6], fruits, such as grapes [7] and strawberries [8], fish products [9], and chewing gum [10].

Our objective will be to determine whether this extract can be used in our daily diet as an organic preservative, to test its impact on the appearance and taste of cheese, and to assess the cost compared with a conventional industrial preservative.

2 Experimental

The first step is to prepare our GSE, which will look like a vegetable powder. The process is simple and consists first of all of extracting the seeds from the fruit, then leaving them to dry in the open air for 14



days, after which they are ground into a fine powder using an electric grinder. The last part consists of filtering the powder to remove the remains of the pericarp (seed coat). The powder is then stored at room temperature away from direct sunlight.

The second step is to prepare the cheese at the company "TAMMY", using the same composition and recipe of the "TIPTOP" processed cheese bar, but at a Laboratory-scale to obtain a 1kg preparation of melted cheese. Once the cheese is prepared we will use a total of 11 samples for analysis. Each sample will contain around 40g of cheese, which will be stored in sterile pots. 2 out of 11 will be control samples, the first one being the positive control that contains the dosage of Potassium sorbate "E202" administered by the company (0.2%) and the second one is the negative control that contains no preservative. For the remaining 9 samples, 3 different dosages (0.5%; 1%; 2%) of each of the 3 GSEs will be added to the cheese (GSE Aroma-Zone, GSE Original Dioter, GSE powder obtained from local grapefruit seeds). We'll then leave the samples in a cool place for 24 hours to allow the grapefruit seed powder to macerate in the cheese and then the tests can begin.

To study the antibacterial and antifungal efficacy of GSE administered to processed cheese, against bacterial and yeast strains. We will test three types of bacteria, After 48 hours of incubation, the GSE with antibacterial and antifungal activity closest to that of the E202-containing control will be used for the next tests. The final tests will be carried out at the company "TAMMY", and will involve measuring the pH of the cheese containing the GSE, its consistency using a penetrometer, and the percentage of dry matter, and comparing these fundamental parameters with those of the TIPTOP control cheese. At the same time, a stability test will be carried out, in which the GSE-based cheeses and the control will be kept in stability chambers at 37°C for 21 days to study their shelf life. We will note the taste, appearance, color and odor of the cheese with 10 people who will testify to see if GSE has an influence on them.

3 Results and Discussion

For the microbiological tests, the sample containing no preservatives logically proliferated the growth of all the micro-organisms. Potassium sorbate showed impressive antifungal activity on *Candida Albicans*, inhibiting around 97% of the yeasts initially present, but surprisingly showed no antibacterial activity on any of the bacteria tested. According to the literature, this is probably due to the pH of the cheese, which is around 5.8, because according to a study carried out in 1962, *S.Aureus* is inhibited by 0.1% sorbate at a pH value of 5. But from a pH ≥ 7 , concentrations of up to 10% seem to have little inhibiting effect [11]. Our GSE was antifungal at a concentration of 2%, but showed no antibacterial activity. Similarly, GSE Original Dioter inhibited all yeasts at a concentration of 2%, and showed a slight spectrum of activity on *E.Coli* at the different concentrations. As for Aroma-Zone's GSE, it showed impeccable antibacterial and antifungal activity at all concentrations, far surpassing the efficacy of potassium sorbate.

Table 1: Results of microbiological tests on the efficacy of GSE on different strains of bacteria and yeast at different concentrations.

<i>Bacteria</i>	<i>Escherichia Coli</i>			<i>Staphylococcus Aureus</i>			<i>Candida Albicans</i>		
	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%
GSE Aroma-Zone	Absence	Absence	Absence	Absence	10	Absence	10	Absence	Absence
GSE Dioter	250	680	350	Ind-Nbr	Ind-Nbr	Ind-Nbr	920	1200	Absence
GSE Powder	Ind-Nbr	Ind-Nbr	Ind-Nbr	Ind-Nbr	Ind-Nbr	Ind-Nbr	1600	820	40
Positive Control (E202)	Ind-Nbr			Ind-Nbr			20		
Negative Control (No Preservative)	Ind-Nbr			Ind-Nbr			1440		

4 Conclusions

Through this study we were able to prove the effectiveness of GSE as a natural preservative as well as its ability to replace E202, we have thus enabled the recovery of these seeds which are often considered as waste by the consumer society. Our experiments have demonstrated the antifungal activity of GSE for the

3 extracts used. Our grapefruit seed powder proved effective at a concentration of 2%, it provided an inhibition spectrum similar to that of E202. The GSE Original Dioter obtained by the hydro-glycerinated maceration process showed no antibacterial activity against *Staphylococcus Aureus*, slight activity against *E.coli*, and total inhibition of *Candida Albicans* at a concentration of 2%. As for GSE Aroma-Zone, it was clearly more effective than E202 on all points, and this at the lowest concentration (0.5%). We have demonstrated that the application of GSE in milk-based products, more specifically cheese, as well as high temperature do not deteriorate the active ingredients contained in GSE. Using a natural ingredient as a preservative will allow manufacturers to display the “Preservative-free” label, while reassuring customers about the conservation of the product.

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