TECHNICAL SPECIFICATIONS

04/08/2021

PRODUCT: PreNat®

Description: 99% dried pomegranate with hot air (<75º C), and micronized (< 300 µ).

1

Challenge test: *L. monocytogenes* and *E. coli* in minced meat with powdered pomegranate peel

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1. Aim

A Challenge Test can provide information on the behavior of certain microorganisms when they are artificially inoculated into a food, under given storage conditions and in the presence or absence of compounds with antimicrobial activity. The aim of this work was to study the antimicrobial effect of powdered pomegranate (**PreNat®**) on the *L. monocytogenes* and *E. coli* growth in chicken minced meat during 11 days at 4º C.

2. Methodology

2.1 Materials

- The chicken minced meat was bought in a local supermarket (Fig. 1).

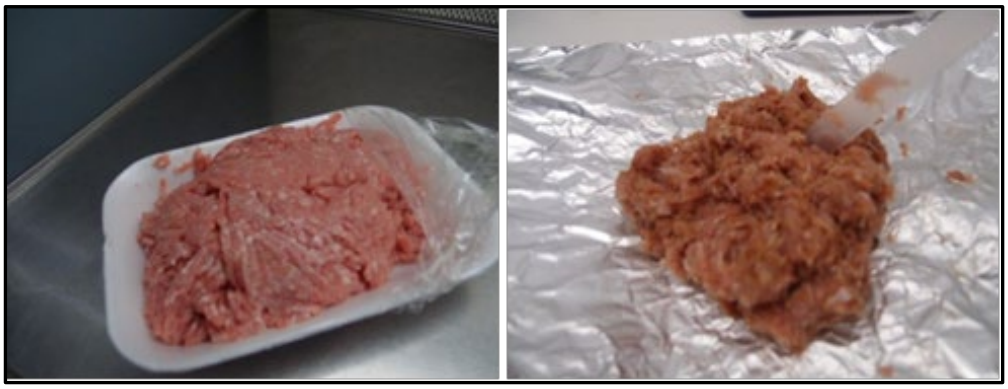

Figure 1. Chicken minced meat used in the study.

- The **PreNat®** used in the experiment is obtained by the drying of pomegranates with hot air. The dry material is pasteurized, then micronized in a grinder and the particle size of the ground product is lower than 300 µm.

- The *L. monocytogenes* y *E. coli* strains (Table 1) were provided by the Spanish Type Culture Collection (CECT).

Table 1. Strains of target microorganisms used in the study.

Code	Strains	Origin	Batch
CRC068	*L. monocytogenes* CIP 59.53 (Murray et al. 1926) Pirie 1940.	CECT 935	20-02-2020
CRC050	*E. coli,* ATCC 25922 (Migula, 1895) Castellani & Chlamers 1919.	CECT 434	13-02-2018

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2.2 Experimental design

Treatments: 1- Control 1 (minced meat without inoculation)

2- Control 2 (inoculated minced meat without **PreNat®**)

3- Treatment 1 (inoculated minced meat with **PreNat®** 2.5%)

Food model product: Chicken minced meat.

Natural antimicrobial: **PreNat®** (2.5%).

Target microorganisms: *L. monocytogenes* and *E. coli* (separated trials)

Number of replications: 3 (per treatment)

Storage temperature: 4º C

Packaging: Vacuum heat-sealed plastic bags.

Sampling for counts: 0, 3, 7 and 11 days.

2.3 Growth prediction using ComBase Browser

The ComBase Predictive Models are a collection of software tools based on ComBase data to predict the growth or inactivation of microorganisms. The ComBase Browser enables you to search thousands of microbial growth and survival curves that have been collated in research establishments and from publications.

It is described how factors such as temperature, pH, and water activity (aw), as well as other conditions (preservatives and atmosphere) affect the growth of different species of microorganisms.

ComBase is a very useful tool for food businesses to carry out safer ways of food production. This includes reformulating and developing new food products, designing challenge testing protocols, developing food safety plans, and helping public health organizations develop science-based food policies through quantitative risk assessment. In this study, this tool is used to verify the evolution of the studied species based on the characteristics of pH, aw and incubation temperature. Taking into account that in some cases the real characteristics are more unfavorable due to the limitations of the ComBase program (for example the case of the storage temperature for the case of *E. coli* where the minimum is 10ºC and on the contrary for the test laboratory meat has been preserved at 4ºC).

2.4 Challenge test: *L. monocytogenes* **and** *E. coli* **in chicken minced meat with PreNat® (2.5%).**

The methods used to carry out the tests were:

- Samples preparation

Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 2: Specific rules for the preparation of meat and meat products (ISO 6887-2:2017).

- *L. monocytogenes* counts

AL Detection validated for the detection of *Listeria* spp. and *L. monocytogenes* **(BRD 07/16-01/09). Reference method:** ISO 11290-1 (2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. - Part 1: detection method.

- *E. coli* counts

RAPID´E. coli 2 validated for the enumeration at 37°C of β-glucuronidase positive *E. coli* **(BRD 07/07-12-04). Reference method:** ISO 16649-2 (2001): Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of B-glucuronidase-positive E. coli - Part 2: colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl B-D-glucuronate.

3. Results

3.1 Growth prediction using ComBase Browser

- *L. monocytogenes.* Conditions for growth prediction

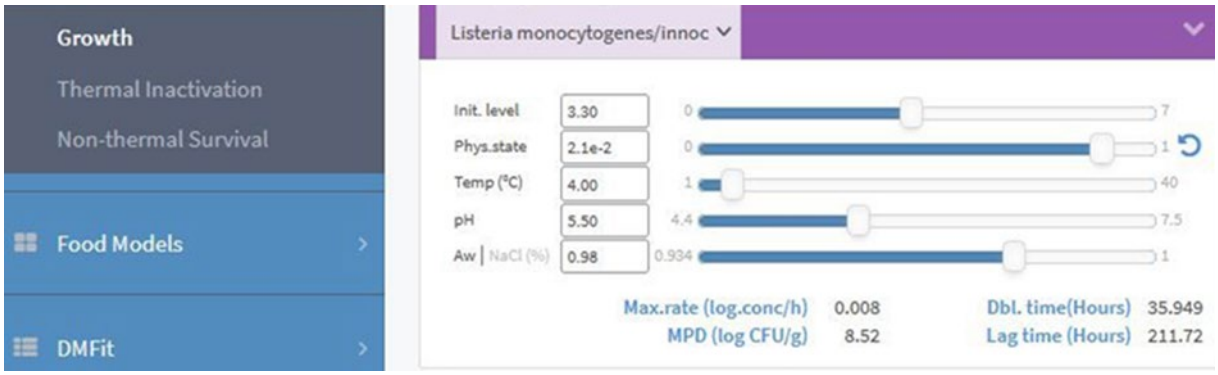
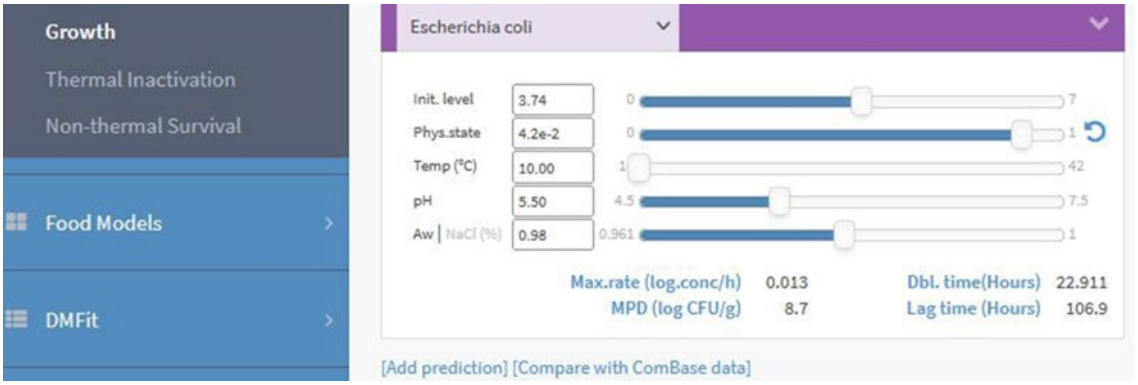


Figure 2. Growth prediction for *L. monocytogenes*.

According to the prediction obtained for growth under the aforementioned conditions, *L. monocytogenes* would reach levels close to 4 log cfu/g after 11 days (264h) at 10ºC.

- *E. coli.* Conditions for growth prediction



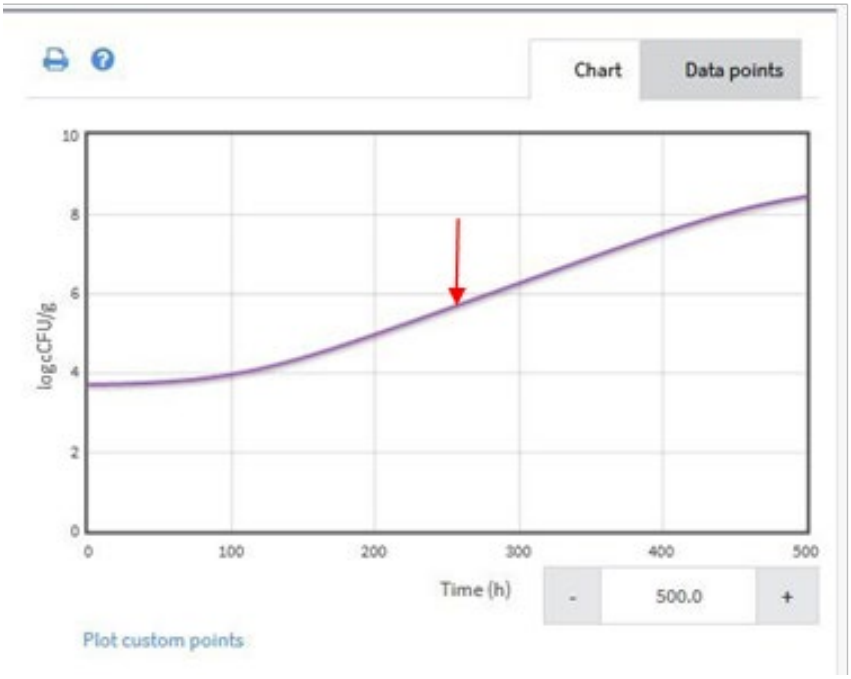


Figure 3. Growth prediction for *E. coli*.

According to the prediction obtained for growth under the aforementioned conditions, *E. coli* would reach levels close to 6 log cfu/g after 11 days (264h) at 10ºC.

3.2 Challenge test: *L. monocytogenes* **and** *E. coli* **in chicken minced meat with PreNat® (2.5%).**

- *L. monocytogenes* growth in minced chicken meat with **PreNat® (2.5%).**

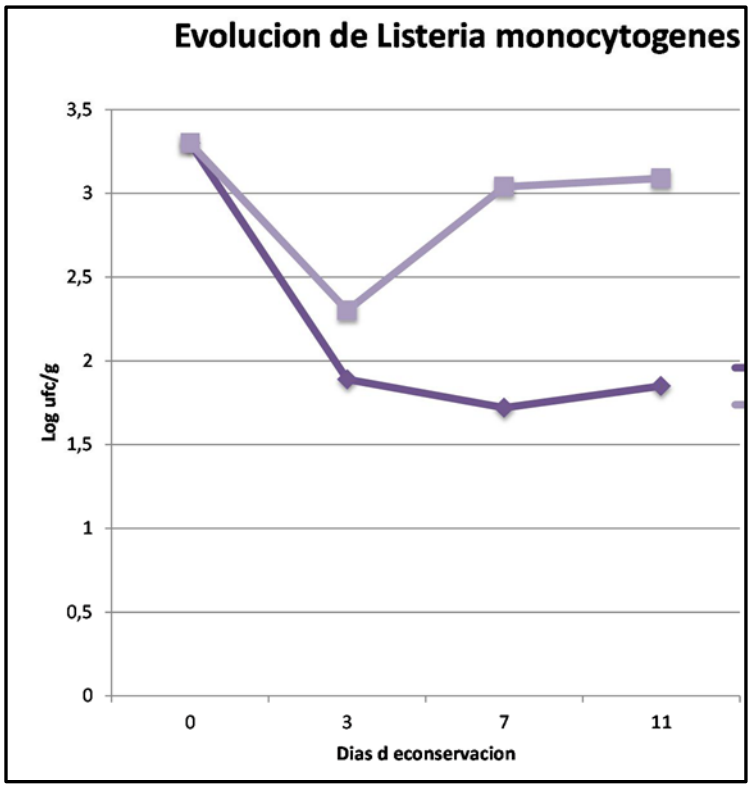


Figure 4. Growth evolution of *L. monocytogenes* in minced chicken meat with (♦) and without (■) **PreNat®** (2.5%).

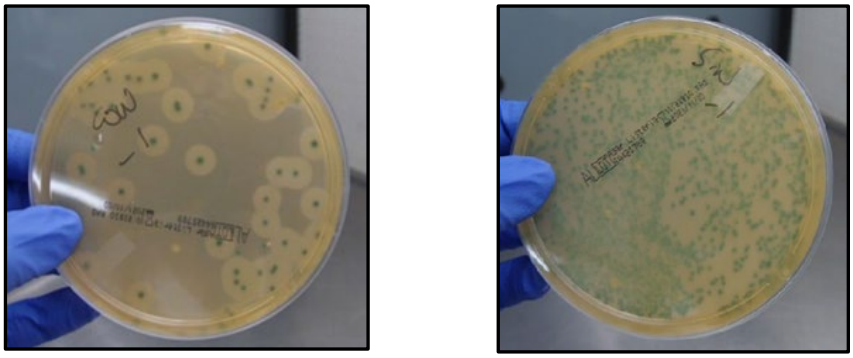


Figure 5. Growth of *L. monocytogenes* in AL Detection culture medium, left (with **PreNat®** (2.5%) and right (without **PreNat®**).

According to laboratory analysis, *L. monocytogenes* counts remain constant close to 3 log cfu/g after 11 days of refrigeration storage in chicken meat samples without **PreNat®** and, on the contrary, decreases to 1.9 log cfu/g when **PreNat®** (2.5%) is used. Under these conditions, the ingredient marketed by Agrosingularity has shown inhibitory capacity.

The growth potential (δ) for *L. monocytogenes* in the case of the meat samples with **PreNat®** (2.5%) is -1.45 and in the case of the control sample without **PreNat®**, -0.21, with the difference between both growth potentials greater than 0.5 , the negative effect that **PreNat®** (2.5%) has on the growth of *L. monocytogenes* in chicken minced meat is significant.

- *E. coli* growth in minced chicken meat with **PreNat®** (2.5%).

According to the analyzes carried out in the laboratory, *E. coli* is able to grow in minced chicken meat from 3.74 to 4 log cfu/g after 11 days in refrigeration (4º C) in the control samples (chicken meat without **PreNat®**). On the contrary, decreases to 2.3 log cfu/g when **PreNat®** is present at the studied concentration (2.5%). Under these conditions, the ingredient marketed by Agrosingularity has shown partial bactericidal capacity.

The growth potential (δ) for *E. coli* in the case of meat samples with **PreNat®** is -1.44 and in the case of the control sample without **PreNat®** is 0.36, with the difference between both growth potentials greater than 0.5, it remains evidenced the negative effect that the presence of **PreNat®** (2.5%) causes on the growth of *E. coli* in chicken minced meat.

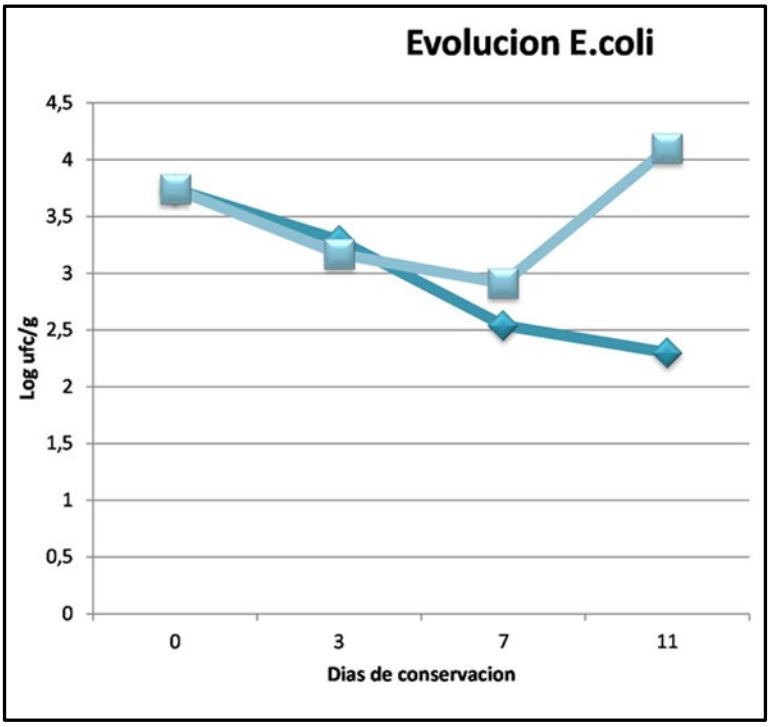


Figure 6. Growth evolution of *E. coli* in minced chicken meat with (♦) and without (■)**PreNat®**.

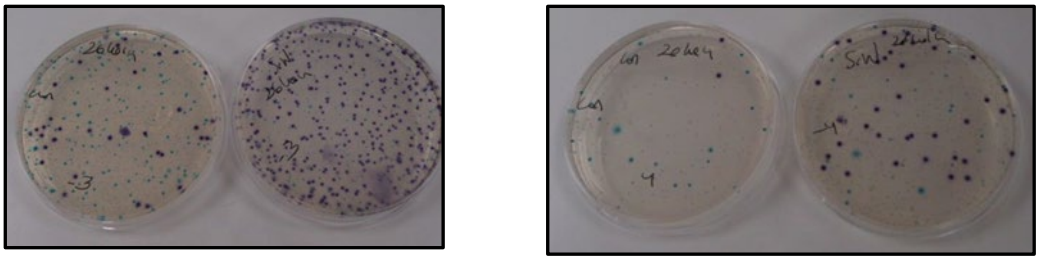


Figure 7. Growth of *E. coli* in RAPID´*E. coli* 2 culture medium, plates left (with) and plates right (without **PreNat®**).

4. Conclusions

Finally, according to the results obtained, we can conclude that after 11 days of storage at 4º C, the presence of **PreNat®** (2.5%) in chicken minced meat, achieves 1.8 log and 1.2 log reductions on the growth of *E. coli* and *L. monocytogenes* respectively.

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Development Manager

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