THE UTILIZATION OF PROPOLIS IN THE MANGO SEMIPREPARATION STABILIZED BY MEANS OF COMBINED METHODS. PRELIMINARY STUDY

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Introduction

The preservation of the products and vegetables by means of „combined factors" is based on the inhibition of the development of bacteria, fungind leculoses, by means of the interaction of the various stress factors: the concentration of water, the pH, the slight termic treatment and others. These factors act more by the reduction or prevention of the growth rather than by killing the microorganisms (1—3).

The preservation of an aliment by means of water concentration is achieved by solving the soluble watery matters of the aliments or by the extraction of water by means of methods like: salting, drying, smoking and liophylization (1—4).

The incorporation of certain weak and lipophylic acids (the citric acid, the benzoic, sorbic and propionic acids), which act as transporters of protons through the cellular membrane, favourizes the acidification of the cell. Moreover, some of them are efficient preservers not only because they are proton conductors, but also because they themselves inhibit the growth when they are inside the microorganism (1). In general, the efficiency of these is higher if the pH is lower, because the molecular form is the one which has the capacity to inhibit the development of the microorganisms (2).

The idea which lays at the basis of the method of preservation by means of combined factors consists of using not only one factor of preservation, but of associating antimicrobial factors so that even when separated, they do not manage to prevent the development of the microbs; in combinations, they increase or/and diminish the request of energy (1).

The main advantages of the preservation of the fruits and of the fruits! pulp, by means of the combined methods are: it does not require a rigorous thermic treatment (sterilization or freezing), but only a simple heating or scaling, with the respective economy of energy; no ermetic containers are needed. This is why the technology is simple and accesible and, consequently, it may also be applied in the developing countries. From the sensory point of view, the products obtained by means of these methods look more like fresh fruits than like dehydrated ones.

Two projects were achieved in the Latin-American Program for Science and Technology and in the CYTED-D Centenary for Development: „the elaboration of aliments with medium humidity important for Latin Ame-

rica" and, more recently, the one referring to the „preservation of the fruits in bulk by means of the method of combined factors", which implies various research works, among which we may mention studies on pulp and on pieces of mango (3, 4, 5, 6) and guayaba (2, 7, 8).

The pulp obtained by the combined methods may constitute a semipreparation for industrial use, utilized in the fabrication of juices, nectars, creams and others.

Considering the necessity of enlarging and diversifying the range of preservation agents in the alimentary industry, the interdiction of their utilization in the aliments for childern and their high cost on the international market, the utilization of other preservers, are interesting. Propolis appears as an attractive alternative, if one considers the fact that it is available and it has a natural origin, which makes him different from the rest of the chemical preserves usually used in industry.

Propolis is a resinous substance elaborated by the bees. It mainly contains beeswax, essential oils and flavonoids; it is soluble in alcohol and in other solvents, such as: ether, acetone and benzene (9—11). It is a heterogenous product, of an unsuspected chemical and biochemical complexity, which makes it have acknowledged bactericidal, bacteriostatic, anaesthetic, antitoxical, antiviral, fungicidal, fungistatic, antiimflammatory and dermoplastic characteristics (11).

Propolis has the advantage of being an unharmful product for man; among others, because of its medicinal qualities, it started to be used for children as well.

The objective of the present report has been that of defining the utilization of propolis as an antimycotic agent in the mango semipreparations, by means of the combined factors technique, and especially the possibility of its utilization to the preparation of the fruit puree for childern.

Materials and Method

Raw materials and testing material

- Sterilized mango pulp preserve, of the Keit type;
- Sugar for industrial use (white);
- 5% red mangrove propolis tincture in 70% alcohol;
- 70% alcohol;
- *Aspergillus niger* inoculate;
- Agar with malt extract;
- Peptonated water;
- Lactic acid 10%;

Procedure

We prepared a mango semipreparate from the preserved pulp, sugar and water in the rations indicated by Table 1.

Table 1

The Formula Experimented with the Mango Semipreparation

Ingredients	%
Preserved mango pulp	85
Commercial sugar	12
Water	3

Table 2 indicates the physical and chemical characteristics noticed in the pure pulp preserve and in the semipreparation (12—14). The water

Table 2

Determinations	Pulp	Semipreparation
Soluble solids (°Bx)	13,5 ± 0,8	23,5 ± 1,2
pH index	3,9 ± 0,2	4,2 ± 0,11
Acidity (expressed as citric acid)	0,5 ±0,17	0,29 ±0,11
Water concentration	0,973 ± 0,004	0,962 ± 0,007

concentration was determined by the instrumental method, in an electronic Novasina hydrometer, model Humidad TH-2 to 30°C.

Then, we added propolis tincture to the semipreparation, in order to obtain a pure propolis content of 5000 ppm. Besides this, we prepared two standard solutions: one with 70% alcohol and another without any additive. The samples were prepared by solving the sugar by means of heating the pulp up to a temperature which is close to the boiling point of water (95°C), and adding the propolis tincture while it was still warm. The sample to which the alcohol was added was left to cool, in order to avoid the evaporation of the alcohol.

The Separation, Identification and Study of the Antimicrobial Action

The mango pulp used for this study was left to go bad at the temperature of the surrounding environment. Subsequently, after 7 days, it was inseminated on the agar with malt extract and the best developed microorganism was prelevated — it was a black fungus.

A wet room was prepared (15) and a small quantity of spores was deposited in it. The spores were incubated at the room temperature and were left there, up to the total development of the fungus, namely until the sporangia became completely black. This process was noticed under the microscope and the observations were compared to the identification catalogue for fungi (16). The result was an *Aspergillus niger,* microorganism which is characteristic of the spoiled fruit pulp.

The fungus was inseminated on agar plates with malt extract, in order that it grow up; subsequently, the spores were collected by means of sterile peptonated water.

The counting of the existing spores was made in a Neubauer 2097 chamber. The number of spores was rounded to about 10^5 by means of successive dilutions, the initial inoculate being of 4.4×10^5 germs/g.

For this alternative preparation, we prepared 100 g samples in sterilized laboratory vessels, also including the respective standards. 1 ml of spores suspension was inoculated in each vessel, they were homogenized and then inseminated at 0.6, 12 and 18 days.

Results and Discussions

Figure 1 shows the results for the microbiel action in the semipreparations which contain propolis, alcohol and no additive.

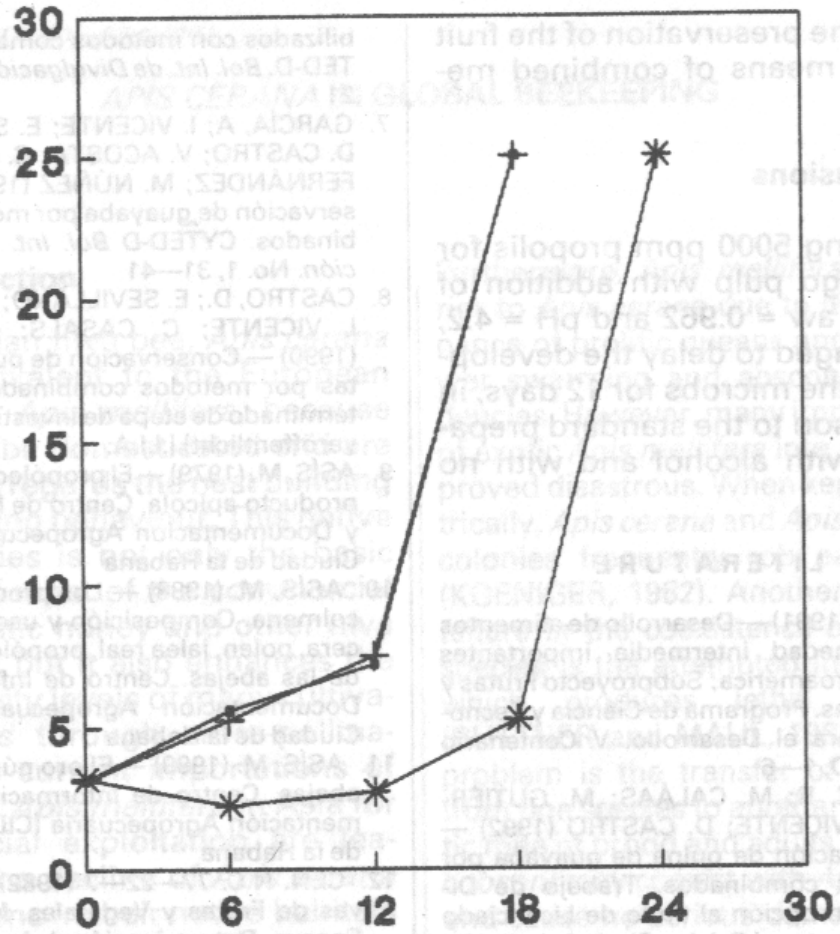

Fig. 1 — The counting of the micobs in the mango pulp alternatives preserved by means of combined methods
Logarithmic counting; uncountable; Time (days); Pulp without additives; Pulp alcohol; Pulp with propolis

As one may see, there was a similar behaviour of the two standards (the one with 6.6% alcohol and the one without additive), and, as time went by, the number of microbs increased, passing from 10^3 to 10^5, in 6 days, and from this value to 10^7 in 12 days, after which the semipreparation became obviously spoiled. It is obsious that the process of delaying in the development of the microbs did not occur and that a logarithmic increase took place all the time.

On the other hand, if the results of the propolis tests are analysed, we may observe the existence of the above mentioned process, but, from the 12th day, the number of microbs begins to increase, reaching, in the 18th day, the same values obtained in the 6 days'standards (10^5). From that moment, no more countings were made, as the decomposition was obvious.

The difference between the results for the propolis semipreparation and those for the standards, demonstrated that propolis delays the growth of *Aspergillus niger* with at least 12 days, fact which suggests that it may be used in combination with the "stress" factors, namely aw = 0.962 and pH = 4.2, thus opening

ves for the preservation of the fruit pulp by means of combined methods.

Conclusions

By using 5000 ppm propolis for the mango pulp with addition of sugar to aw = 0.962 and pH = 4.2, we managed to delay the development of the microbs for 12 days, in comparison to the standard preparations with alcohol and with no additive.

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