Nor. Afr. J. Food Nutr. Res. 2021; 5(11): 10-14

https://doi.org/10.51745/najfnr.5.11.10-14

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ORIGINAL ARTICLE

Mold Chitosan production using *Ficus microcarpa* fruit

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Abstract

Aims: The main objective of this work was the valorization of *Ficus microcarpa* fruit, an inedible fruit with a high nutritional value in the production of chitosan by *Aspergillus niger*. **Methods:** A surface and submerged growth of *Aspergillus niger* were carried out using the *Ficus microcarpa* fruit syrup. 3 106 spores/ 50 ml were inoculated. Chitosan production was conducted at 37° C for 14 days using both surface and submerged fermentation modes. The growth of *Aspergillus niger* was followed by spore count on a Malassez cell. The evolution of pH and the consumption of total sugars in the mixture were monitored. Chitosan was extracted after degumming with NaOH 10N and centrifugation. **Results:** During the first days, the fungal growth was exponential with maximum growth rates of 10.06 g/L/day and 12.05g/L/day respectively for the surface and submerged cultures. The mycelial growth was largely linked to a high intake of sugars. The chitosan production in glucose syrup was significantly lower than the production using *Ficus microcarpa* syrup. This syrup allowed producing 41 g/L of chitosan for the surface culture and 37.2g/L for the submerged culture. Infrared analysis of chitosan powders showed similar spectra to those of commercial and shrimp chitosan. This first work is a contribution to a better valorization of a bioresource.

Keywords: *Aspergillus niger*, culture, chitosan, *Ficus microcarpa* fruit, syrup.

Received: December 10, 2020 / **Accepted**: February 17, 2021 / **Published**: March 4, 2021

1 Introduction

Chitosan, a natural and biodegradable biopolymer, is produced from crustaceans such as crab, shrimp, lobster and crayfish. This polymer constitutes the most abundant polysaccharide in the world after cellulose. However, production of chitosan from shellfish is limited by the seasons and the fishing industry. Chitosan is known to be present as well as the shrimp antigen in the final product [1].

Chitosan arouses greater interest than chitin, given the greater number of amine groups it contains. It is a good candidate for many applications in various fields, namely medicine, pharmacology and water treatment [2,3].

Chitosan could be produced by fungal mycelium by simple fermentation regardless of geographic location or season [4].

For industrial applications, *Aspergillus niger* is the best choice due to its easy development, genetic stability, high yields, and low production of undesirable metabolites [5]. This mold is responsible for the degradation and synthesis of organic acids such as citric acid [6] and other compounds that could be used in biotechnology, agriculture, food industry, and environment [7].

Ficus microcarpa is a tree native from tropical Asia and grows spontaneously throughout the Mediterranean Sea. Cultivated for ornamental purposes, some fig trees can grow to over 30 meters in height and produce very small, non-edible fruit [8]. This small-sized fruit (Figure 1.a) is characterized by a nearly rounded shape with a thickness of 11.9789 ± 0.2 mm, 1.169 ± 0.5 cm in diameter and 1.477 g ± 0.01 g of weight. The pulp is composed of achenes (Figure 1.b). This fruit is inedible and constitutes a source of minerals (Ca and K), sugars and essential fatty acids (oleic, palmitic) and bioactive substances such as saponins, which have been used to prepare a scar removal ointment [9].

To the best of our knowledge, no prior studies have been published on the mold production of chitosan using *F. microcarpa* fruit syrup.

The main objective of this study was to produce chitosan by *A. niger* using *F. microcarpa* fruit syrup as a substrate.

2 Materials and Methods

2.1 Plant material

The *Ficus microcarpa* fruit was used as a substrate for this study. Fruits were harvested in the urban settings of the town of Boumerdes (Northern Algeria) from October to November 2016. The fruit was sorted, washed with tap water, and then transformed into syrup and stored at 4 °C in a refrigerator before use.

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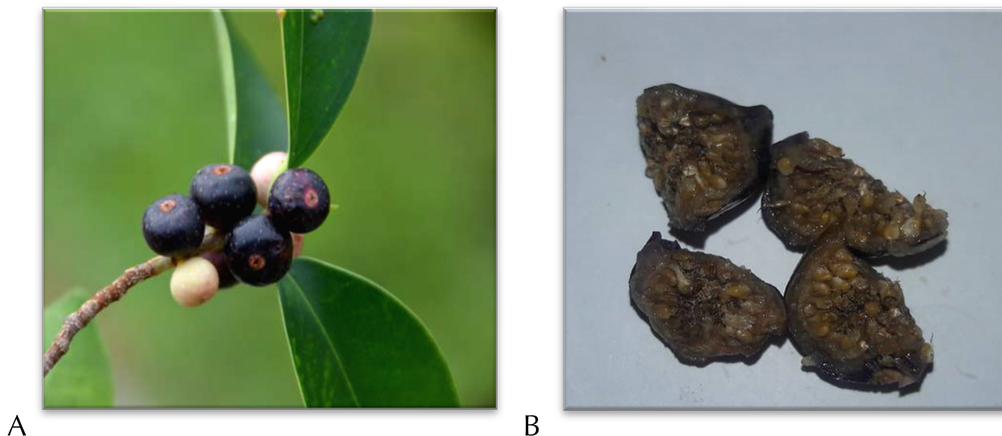

Figure 1: *Ficus microcarpa* fruit A: mature and immature fruit, B: pulp and achenes

2.2 Biological material

The mold strain used for the chitosan production is *Aspergillus niger* isolated from an orange rot in the Engineering Microbiology Laboratory of Mouloud Mammeri University of Tizi-Ouzou. The strain was purified and then stored at 4 °C on OGA Agar.

The mold purification was carried out five times on the same medium to obtain identical colonies [10].

The spores of this strain were grown on the same medium with chloramphenicol at 22 C° for 7 days.

2.3 Methods

2.3.1 Syrups preparation

The *F. microcarpa* fruits were washed and cut into small pieces using a blender; the ground material was soaked in water according to (1:3) (g/g) fruit/water ratio, then the mixture was heated to 65°C in a water bath for 3 h with agitation. The filtration was performed to extract maximum amounts of phytochemical compounds.

Glucose syrup and *F. microcarpa* fruit syrup were prepared in the same Brix percentage (5%).

They were sterilized by tyndallization and then stored at 4°C before fermentation to avoid bioconversion of fermentable sugars.

Table 1: Biochemical characteristics of the prepared syrups

	Ficus microcarpa fruit syrup	Glucose syrup
pH	5.8±0.025	6.75±0.015
Brix (%)	5	5
Total Sugars (g/100ml)	67.80±0.025	33.61±0.03
Reducing Sugars (g/100ml)	13.81±0.025	32.54±0.03
Titratable acidity (%)	2±0.002	0.8±0.0013

2.3.2 Fungal chitosan production

The initial concentration of *A. niger* spores used to inoculate both syrups was 3 106 spores/50 ml of syrup. The inoculation was carried out with a ratio of (50/300) (v/v) inoculum/syrup according to Zergat, [10]. The chitosan bioconversion was conducted at 37 °C for 14 days using both surface and submerged fermentation modes to obtain maximum chitosan yield. Indeed, the ideal temperature for the growth of *Aspergillus* is between 20 and 42 °C [11].

2.3.3 Growth parameters

- **Spore account**
  The method of spore count on a Malassez cell was performed using an optical microscope (Notic SFC- 18 series).
- **pH measurement**
  The direct measurement of pH was performed using a pH meter (INOLAB).
- **Determination of sugars**
  Total and reducing sugars were determined according to the method of Dubois *et al.* [12] using Fehling solutions. Sugar is the essential substrate for the growth of *A. niger*, and the evolution of its concentration in the growing medium gives an indication of its growth rate.
- **Determination of mycelium growth rate**
  The mycelial growth rate was calculated using the following equation:

  Mycelial growth = $dx/dt = (X-X_0)/(t-t_0)$
  Where:
  X = dry mycelium weight at t
  X_0 = dry mycelium weight at t_0
  Mycelia growth rate (g/L/Day)

2.3.4 Chitosan extraction and characterization

The Extraction of chitosan from fungal biomass was conducted as described by Aghdam [13]. The extraction protocol is illustrated in figure 2.

The functional groups of the obtained chitosan were investigated with IR spectrometry (BRUKE ALPHA, China).

The UV-Visible spectrum of chitosan solution (5mg/100ml HCl) was determined using UV-Visible spectrophotometer (Medline).

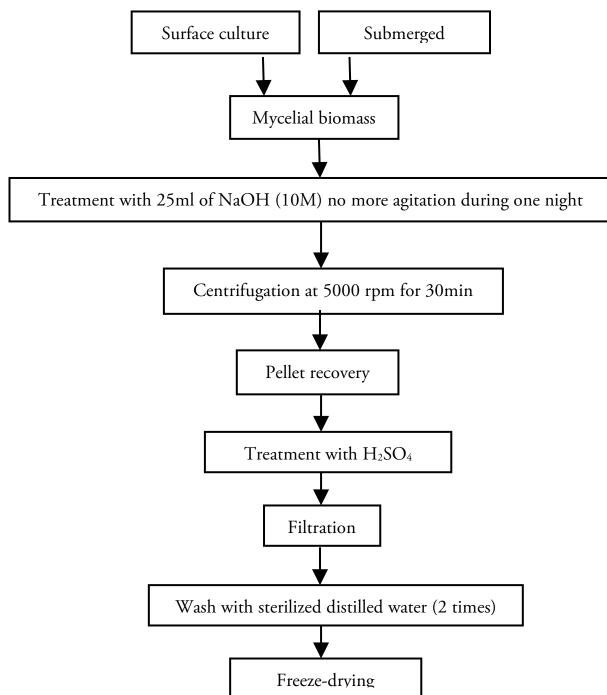

Figure 2: Chemical extraction of chitosan from fungal biomass

2.4 Statistical analysis

Three replicates per treatment were used to assess all the parameters analyzed (pH, Total Sugars, Mycelial growth, etc.).

3 Results and discussion

3.1 Growth kinetic of *Aspergillus niger* mycelium

The main results show that *Aspergillus niger* strain developed only in *Ficus microcarpa* syrup and not on glucose solution. This may be due to the lack of certain components that are present in *F. microcarpa* syrup but absent in glucose solution such as minerals and nitrogen.

Mattey [14] and Pagianni [15] have shown that increased mycelial growth and sugar uptake are associated with elevated nitrogen and phosphorus concentrations.

The evolution curves of the rates of mycelial growth, sugars consumption and pH in the two cultures (surface and submerged) using the *F.microcarpa* syrup as substrate are presented in Figures 3, 4 and 5. The dry weight evolves gradually to reach its maximum at the end of fermentation, i.e. 41 g/L for the surface culture and 37.2 g/L for the submerged culture.

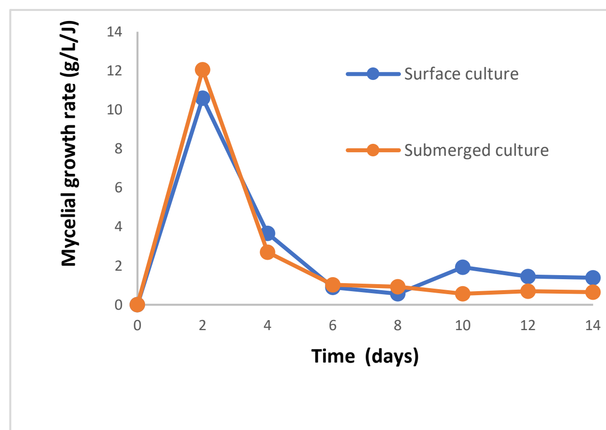

Figure 3: Evolution of mycelial growth rate over time in *F. microcarpa*. fruit syrup medium in the two cultivation methods

Mycelial growth takes place in 4 phases: Since the latency phase is not apparent, we believe this may be attributed to the use of pre-germinated spores.

During the first stage, which lasts 02 days, the mycelium growth is exponential. At this stage the growth rates of the mycelium reach their maximum, i.e.: 10.06 g/L/d and 12.05 g/L/d for the surface culture and submerged culture, respectively. This growth is linked to a high intake of sugars.

The second stage is characterized by a drop in growth rates, which reach 0.56 g/L/d after 8 days for the surface culture and 0.93 g/L/d after 8 days for the submerged culture. This stage, which lasts 10 days, is characterized by growth inhibition due to the lowering of pH (near 4.5) (Figure 5). During the 3rd stage, growth rates increase again to attain 1.45 g/L/d and 0.69 on the twelfth day for the surface culture and the submerged culture, respectively. The fourth stage lasts 2 days, during which, the fungus reduces its growth rate. This rate reaches 1.39 g/L/d and 0.64 g/L/d at the end of the fermentation, for the surface culture and the submerged culture, respectively.

These results are in agreement with those reported by Synowiecki and Al-Khateeb [16]. These authors have shown that this pH promotes optimal fungal production of chitosan (10 mg/50ml) using *Rhizopus*. Indeed, a pH from 4.5 to 5.5 promotes the production of deacetylase chitin, which converts chitin into chitosan in the fungal cell wall [17].

F. microcarpa fruit syrup promotes chitosan production of 32.55±5.6mg/g for surface culture and 19.62±3.6mg/g for submerged culture.

3.2 Functional group of the obtained chitosan

IR analysis (Figure 6) of the chitosan extracted from the mycelial biomass produced by surface and submerged culture shows the same functional groups but with varying intensities.

Similarly, the absorptions observed around (3448.34 cm^{-1} and 3442.48 cm^{-1}) are assigned to primary amine (N-H). Besides, the

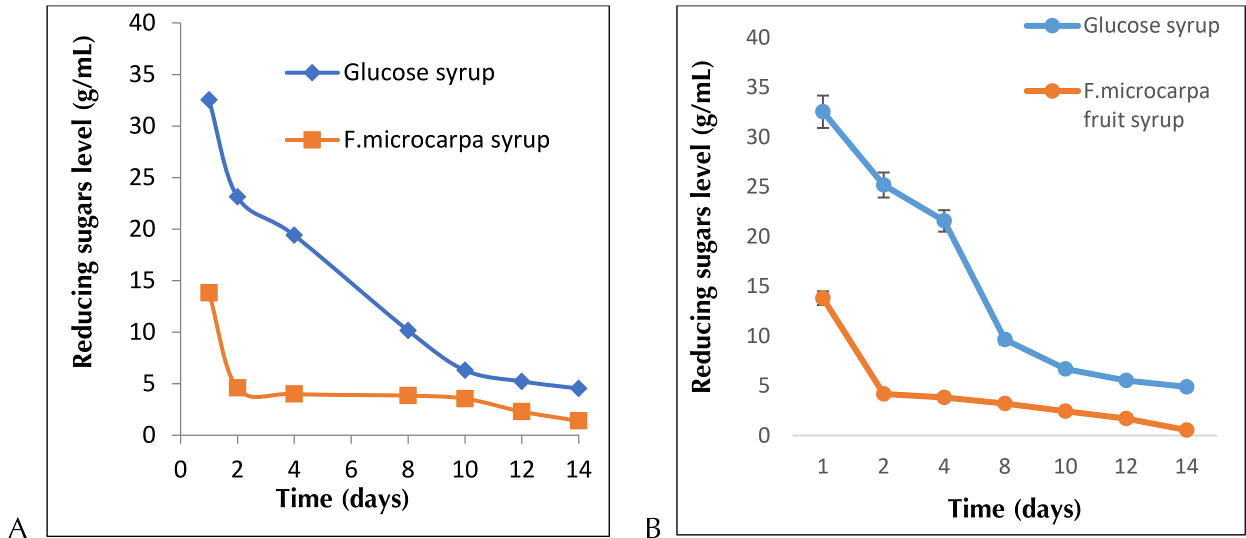

Figure 4: Evolution of reducing sugars over time in the two cultivation methods adopted (A: Submerged culture, B: Surface culture)

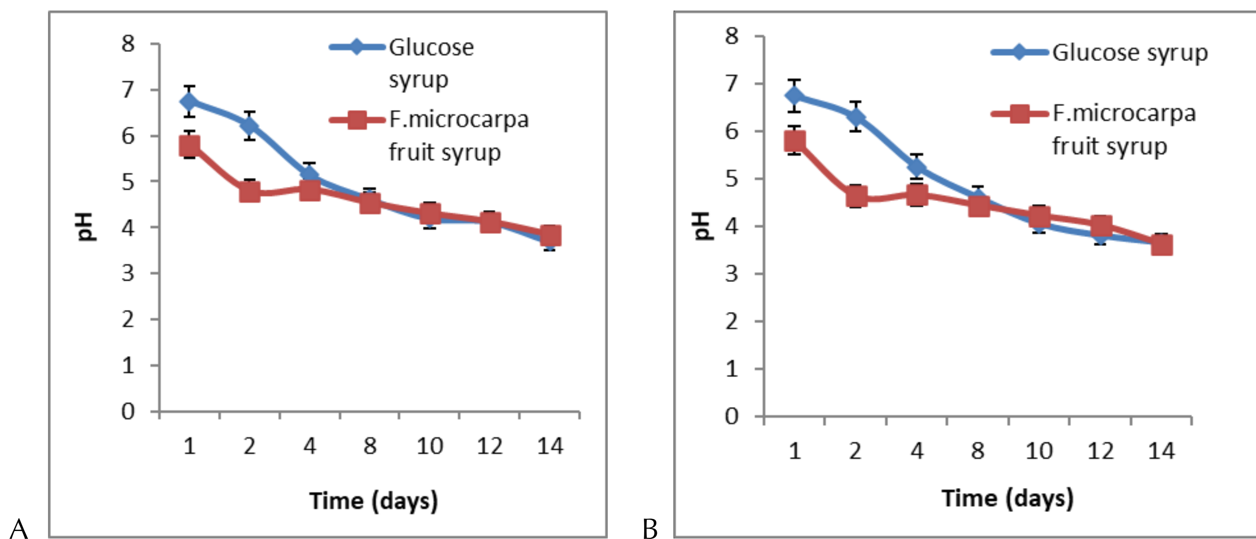

Figure 5: pH evolution as a function of time in the two cultivation methods adopted (A: Submerged culture, B: Surface culture)

signals at 1646.06 and 1241-1640.36 cm^{-1} demonstrate the presence of amide group. Generally, the bands observed between 1559.16 and 1558.60 cm^{-1} correspond more specifically to the elongation vibration of the C-NO2 nitro aliphatic group whereas the signals at 653.78 and 651.84 cm^{-1} demonstrate the presence of the deformation vibration of the C-H group.

The two spectra reveal the presence of several compounds in the composition of chitosan. This result is confirmed by Fig. 7, which demonstrates the absorption spectra of chitosan obtained from both types of culture (surface and submerged). As shown by this figure, the two chitosan absorb at a similar absorption maximum at 340 nm but at different absorbances, 177 and 120 for surface and submerged cultures, respectively. These chitosan samples are very close to those produced by *A.niger* ITCC7635.09 according to Wu *et al.* [18].

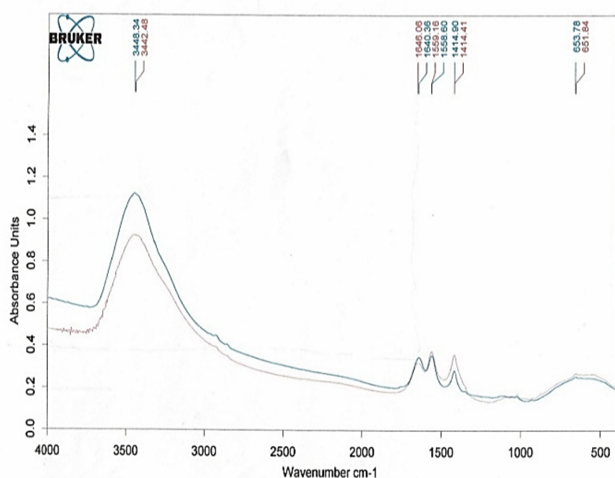

Figure 6: IR spectrum of chitosan extracted from *A. niger* mycelial produced by surface culture (red color) and submerged culture (blue color)

4 Conclusion

We can conclude that the syrup of *F. microcarpa* is a good substrate for the culture of *A. niger* producing chitosan. It would be interesting to conduct research on the kinetics of the main processes and the determination of biological and pharmacological activities of the obtained chitosan.

Author contribution: A.B.D. conducted the experiments, drafted and revised the manuscript. H.B drafted, corrected and revised the manuscript.
A.B. revised the manuscript, Software. All authors approved the final version of the manuscript.

Funding: This work was supported by grants from: Laboratory of Chemistry and Microbiology of Mouloud Mammeri University of Tizi-Ouzou (Algeria), Laboratory of the Research Unit, Materials, Processes & Environment (UR-MPE) of the University of Boumerdes.

Conflict of interest: The authors declare no conflicts of interest.

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Cite this article as: Benahmed Djilali, A., Boughellout, H., & Benseddik, A. (2021). Mold Chitosan production using *Ficus microcarpa* fruit. *The North African Journal of Food and Nutrition Research,* 5(11): 10-14. https://doi.org/10.51745/najfnr.5.11.10-14

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