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Theoretical Foundations and Analysis of the Jerusalem Tubers

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Abstract. Crisply gathered Jerusalem tubers contain inulinase, a protein that requires inactivation, since of its capacity to hydrolysis inulin into fructose, which can be devoured by microorganism amid marinating. As the traditional pickling process takes 6 months, and involves the addition of a large amount of salt (18–20%), this production strategy is uneconomical and increases the nitrite intake. Additionally, miscellaneous bacteria produced during pickling affect the product taste. In this study, the enzyme inactivation effects of NaCl, NaHCO3, and ultrasound were evaluated. NaHCO3 treatment results in the highest degree of enzyme inactivation; however, the quality and flavor of the obtained Jerusalem tubers pickles were not ideal. The Jerusalem tubers pickles in which the enzymes were inactivated using a combination of NaCl and ultrasound exhibited better flavor than those exposed to NaHCO3; further, this combination reduced the inulinase activity of the Jerusalem tubers to 2.50 U/mL, and maintained the inulin content at 61.22%. The strains LS3 and YS2, identified as Enterococcus facials and the salt-tolerant yeast respectively, were the dominant microorganisms isolated from the pickle juice. Jerusalem tubers with inactivated inulinase were pickled with microbial powder, separated, purified, and dried to remove the natural Jerusalem tubers sauce. This process shortened the fermentation cycle and improved product quality [1].

Keywords: functional food, inulinase, bioactive ingredients, enzyme inactivation, microbial powder, inulin

Introduction. Pickled vegetables undergo a traditional microbial fermentation process that employs the preservative effect of salt to prolong the shelf life of the vegetable. The popularity of pickled vegetables has been steadily rising, owing to their unique color, aroma, and low cost. For instance, the unique sensory properties and the potential health benefits associated with lactic acid fermentation in the produced in Yunnan—considered the most authentic—have made it popular in China.

Another pickled product is world-renowned for being rich in vitamin A, thiamine, riboflavin, calcium, iron, and lactic acid-producing bacteria. In addition, several studies have reported that fermented soybean products possess beneficial properties, including antioxidant, antimicrobial, blood pressure-lowering, and ant diabetic activity. Jerusalem tubers pickles are crisp, fragrant, slightly sweet, and tender; further, they are easy to store, which is why they are favored by consumers. Healthier and low-salt pickles are being prepared to match the improved living standards [2]. Moreover, Jerusalem tubers are rich in inulin, which is the second most-common plant storage carbohydrate after starch and accounts for ~50–70% of the Jerusalem tubers tuber stem weight. Inulin is a linear polysaccharide in which D-fructofurans are linked by a β (1→2) bond, with a D-glucose residue—typically residing at the end of individual fructose chains—being linked to fructose by an α (1→2) bond. Thus, the development of Jerusalem tubers pickled products is of economic importance; however, the enzymes involved in the pickling process may alter the flavor and texture of the product. One problem that is encountered during the picking of Jerusalem tubers is the presence of inulinase. During the pickling of fresh Jerusalem tubers, inulin present in these vegetables can be acted on by inulinase. Inulinase can hydrolyze the β (1→2) glycoside bonds between the fructose moieties of inulin, and this process is widely used in the production of oligosaccharides and high-fructose syrup. Inulin can be degraded by inulinase into fructose, which is consumed by microorganism during pickling. Furthermore, inulinase can alter the quality of pickled products, so it is necessary to take appropriate measures to check

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inulinase activity in Jerusalem tubers pickles during the curing process. This not only preserves the beneficial health properties of inulin, but also protects Jerusalem tubers from putrefaction and deterioration, and inhibits discoloration, flavor change, and nutrient content reduction due to enzyme activity [3].

Fig.1.Tubers of Jerusalem artichoke.

Another problem in the processing of Jerusalem tubers pickles is the long curing period, during which a large amount of salt is added (18–20%) and the pickles are exposed to an environment with high nitrite levels and infectious microbes. Notably, the procedure is uneconomical, and the high levels of nitrite and miscellaneous microorganisms produced during the pickling process affect the taste of the product. In this study, dry powders of lactic acid bacteria and yeast strains were separated from pickle juice samples that had been obtained from a pickle factory and used as experimental strains. Microorganisms (mainly lactic acid bacteria and yeasts) play a pivotal role in pickling and affect the quality and safety of the final product [4]. The inulin and nitrite contents of the Jerusalem tubers subjected to traditional and improved pickling processes were determined, with the goal of screening and identifying naturally-brewed strains and shortening the pickling period. Inulinase inactivation in Jerusalem tubers pickles presents many challenges, including the prevention of inulin degradation and preservation of the taste of pickles, while ensuring the production of high-quality Jerusalem tubers pickles. To our knowledge, no method has been developed to inactivate the inulinase to ensure inulin preservation—during the production of Jerusalem tubers pickles. Therefore, it is necessary to prepare high-quality pickles with the raw material of Jerusalem tubers and explore inulinase inactivation, so as to maintain the inulin content of Jerusalem during curing, thereby preserving the health benefits associated with tubers. Specifically, the aim of this study was to investigate the effects of enzyme inactivation during the pickling of Jerusalem tubers. The effect of three different treatments, NaCl, NaHCO3, and ultrasound on inulinase inactivation during Jerusalem tubers pickling were evaluated; their effects on the sensory qualities of the pickles were studied. Then, Jerusalem tubers with inactivated inulinase were pickled using microbial powder, which had been separated, purified, and dried from the natural Jerusalem tubers sauce. This process shortened the fermentation cycle and improved the product quality. Efforts are underway to develop nutritious, therapeutic, low salt, and naturally preserved vegetables [5].

Materials and methods. The tubers were washed, dried, peeled, and weighed. Inulinase activity was determined by preparing inulinase crude extract from 10 g
of fresh Jerusalem tubers tuber. The remaining Jerusalem tubers were cut into thin slices and then heated in boiling water at 100 ℃ for 5–10 min to inactivate polyphenol oxidase (PPO), which is responsible for browning in most
vegetables. Then, the tubers slices were dried in an oven at constant temperature, and the weight was recorded. Jerusalem tubers powder (ground using a grinder and then sifted through a 40-mesh sieve) was mixed with distilled water at a ratio of 1:8 and incubated in a 70◦C water bath for 2 h. The pH was adjusted to 10.0 with lime milk, and the solution was then incubated in an 80◦C water bath for 1 h. Inulin extract was obtained after removing the filter residue using eight layers of gauze. The total sugar and reducing sugar content were determined to calculate the inulin content of the Jerusalem tubers. Fresh Jerusalem tubers were

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washed, dried, sliced into 5 mm-thick slices weighing 100 g, placed in 600 mL pickle jars, and salted with NaCl. The fresh Jerusalem tubers contained 80% water. After salt was added, the Jerusalem tubers slices exuded water to dissolve the salt, and the exudate covered all of the pickled Jerusalem tubers slices. The taste of Jerusalem tubers pickles can change in the presence of excessive salt and nitrite, and the traditional pickle marinade of 18–20% (w/w) salt are not healthy [6].

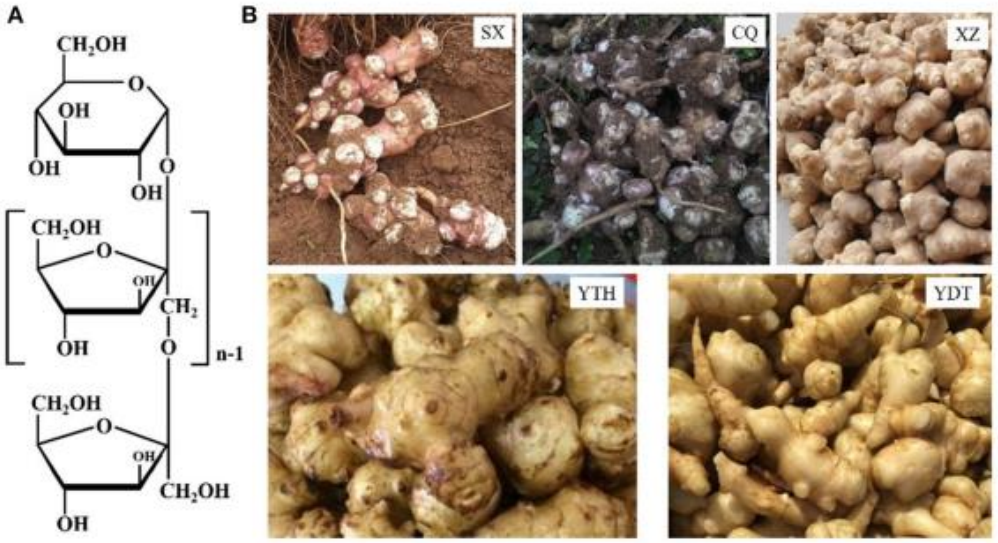

Fig.2 The chemical structure of inulin

Therefore, the concentration of salt was reduced to 10–16% (w/w) in this study, which is cost-effective and benefits the green economy. Jerusalem tubers slices (10 g) pretreated with salt were blended with 50 mL chilled phosphate buffer. The supernatant was collected after centrifugation at 5,000 r/min for 10 min and treated as a crude enzyme to determine inulinase activity. Crude inulinase solution (1 mL) was reacted with of 2% inulin (4 mL) prepared in an acetate buffer (pH 4.5) in a constanttemperature water bath (set at 55◦C) for 30 min, and the reaction was terminated immediately by shifting the contents to a boiling water bath for 5 min. Pre-inactivated inulinase was used as a blank. DNS was used to measure reducing sugar content, and 1 mL of the sample was thoroughly mixed with 3 mL DNS reagent, and incubated in a boiling water bath for 5 min to allow color development. Then, the solution was diluted with distilled water to an appropriate concentration, and the absorbance was measured at a wavelength of 520 nm [7].

Under certain conditions, enzyme activity (U/mL) was defined as the amount of enzyme required to hydrolyze substrates to 1 g of fructose/min in a volume of 1 mL and calculated as follows.

$$E=\frac{1000*C*N}{T*V}$$

E, inulinase activity (U/mL); C, fructose content (mg/mL) corresponding to the average absorption value of the sample aligned with the standard curve; N, dilution of crude inulinase solution; T, reaction time (min); V, crude enzyme volume involved in the reaction (mL). Jerusalem tubers are native to temperate regions of North America and can tolerate an annual precipitation ranging from 31to 282cm, with suitable average temperature range of 6.3–26.6 ℃, and pH of 4.5–8.2. Although it can adapt well to a wide range of soil types and pH levels in a sunny position, slightly alkaline soils are favorable for tubers production. Generally the plant can tolerate sub-zero temperatures while the tubers can withstand freezing for several months even if the frost kills the stems and leaves. The cold-tolerant nature of the tubers allows them to be preserved in the ground during the cold winter until harvested as required. Several studies suggested that Jerusalem tubers should be planted in early spring to a depth of 10–15 cm. Seed tubers should be spaced 30–60cm apart in each row, with rows 45–120cm apart. The optimal soil temperature for planting is between 6 and 7 ℃ due to the fact that tubers become dormant at temperatures lower than 5 ℃.

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Fig.3. Plant of Jerusalem tubers.

In addition to functional foods derived from Jerusalem tubers, the leaves also have important applications. Jerusalem tubers leaves are traditionally used as a folk medicine for the treatment of bone fractures, skin wounds, swelling and pain.

A number of valuable bioactive compounds of medicinal significance have been isolated from the aerial parts of Jerusalem tubers, demonstrating antifungal, antioxidant, anticancer activities and other medicinal effects. Simultaneous scarification and fermentation (SSF) is characterized as inulin hydrolysis and sugar fermentation being carried out in one bioreactor using combination biocatalysts. Obviously, such a direct conversion of soluble inulin into ethanol without a prior hydrolysis step is highly favorable from capital investment and operating cost perspectives. Moreover, a SSF process significantly reduces the fermentable sugar loss caused by separation and transfer of sugars from hydrolyzer into fermenter as in a SHF process. For Jerusalem tubers ethanol production through SSF, the major technical barrier is the identification of the most efficient enzymes which are capable of facilitating both hydrolysis and fermentation. Ultrasonic waves are mechanical vibrations that can alter or accelerate changes in material performance, state, structure, and organization. Currently, ultrasonic processing is widely used as a new no thermal technology for enzyme inactivation. Jerusalem artichoke inulinase could be inactivated by ultrasound, with an optimum [8].

effect at an ultrasonic time of 30–40 min. Ultrasonic treatment was most effective against inulinase from Jerusalem artichoke tubers from SX, with the inulinase activity decreasing from 5.17 to 4.02 U/mL. When the ultra-sonication time was extended to 50–60 min, enzyme activity increased slightly from different regions is irregular, with corners unwashed. Ultrasound can be used to deeply clean Jerusalem artichokes and inactivate inulinase, and it can be integrated with PPO enzyme inactivation, thereby reducing browning in the pickling process.

Conclusion. In this study, we identified a feasible strategy for inactivating of Jerusalem tubers inulinase. The inulinase in Jerusalem tubers pickles was inactivated by employing a combination of salt stress and ultrasonic treatment, without any pronounced deterioration or color changes. Ultrasound for 30–40 min combined with 10% NaCl was found to be the best treatment to effectively deactivate inulinase while still producing a high-quality and flavorful pickle. Furthermore, two Jerusalem tubers pickling strains were identified. A dry powder composed of lactic acid bacteria and yeast strain isolated from pickle juice samples obtained from a pickle factory were used to pickle Jerusalem tubers with inactivated inulinase.

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Date of Publication:30-05-2022

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