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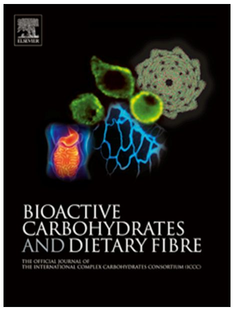

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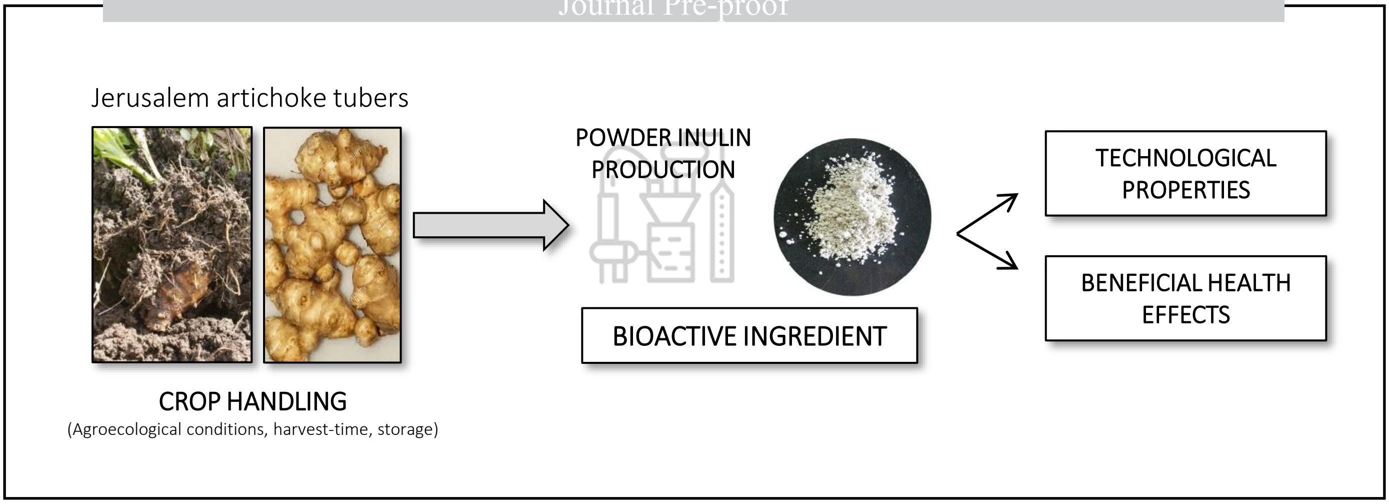
Jerusalem artichoke tubers
CROP HANDLING
(Agroecological conditions, harvest-time, storage)
POWDER INULIN PRODUCTION
BIOACTIVE INGREDIENT
TECHNOLOGICAL PROPERTIES
BENEFICIAL HEALTH EFFECTS

Inulin from Jerusalem artichoke (*Helianthus tuberosus* L.): from its biosynthesis to its application as bioactive ingredient

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Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) represents a promising crop emerging in different parts of the world as a natural source of inulin. Different factors such as the kind of cultivar, agroecological conditions, harvest time, and tubers storage, have an impact on the inulin content and the physicochemical and biological characteristics. A wide variety of protocols for the extraction of inulin from Jerusalem artichoke tubers have been described that should be applied and selected considering the desired purity, the equipment available and the environmental impact. The biosynthesis of the inulin during the plan life cycle, the beneficial health effects of Jerusalem artichoke tubers as well as the application of inulin as bioactive ingredient in functional foods, are presented in this review. The data analyzed reveled that information is missing about the physicochemical characteristics of the inulin used in the different studies. Finally, the reviewed information contributes to the knowledge of the use of this compound as an ingredient in the food industry considering both its technological and bioactive effects.

Keywords: Jerusalem artichoke (*Helianthus tuberosus* L.), inulin biosynthesis, inulin production process, biological activity, technological properties, bioactive ingredient.

Abbreviations

JA: Jerusalem artichoke

JAT: Jerusalem artichoke tubers

DP: Degree of polymerization

FOS: Fructooligosaccharides

Glc: Glucose

Fru: Fructose

Suc: Sucrose

1. Introduction

Fructans are oligo- and polysaccharides that consist of chains of fructose units linked through β (2→1) linkages with a single D-glucosyl unit at the nonreducing end (Panchev, Delchev, Kovacheva, & Slavov, 2011). Considering the degree of polymerization (DP), fructans with a chain length of 2-9 units are generally referred to as fructooligosaccharides (FOS) or oligofructose, and those with longer chain (DP> 10) are called inulin (Apolinário et al., 2014). Because of the particular-linkage configuration between fructose monomers, inulin-type fructans are not degraded by human digestive enzymes, and exert different beneficial physiological effects (Bach, Jensen, Clausen, Bertram, & Edelenbos, 2013; Causey et al., 2000; Li et al., 2013). In particular, the prebiotic activity of inulin-type fructans has been widely demonstrated in many animal models and human nutrition intervention trials (Biedrzycka & Bielecka, 2004; Ramnani et al., 2010; Taper & Roberfroid, 2002).

Fructans are synthesized in leaves of several plants and accumulate in stems and roots, as carbon source reserve, as heterogeneous mixtures with different DP and chemical structures. In higher plants, five types of fructans with different structures were described: inulin-type fructans (1-kestose), levan-type fructans (6-kestose), fructans of the inulin neoseries (neokestose), mixed-type levans (bifurcose), and fructans of the levan neoseries also called mixed-type levans (mixed-type F3 fructan) (Apolinário et al., 2014). These fructans structures are essentially linear; however, a low degree of branching can occur in inulin, through β-(2→6) linkages. In some cases, the terminal glucose molecule may be absent (Fn-type fructans), and so a reducing behavior is observed. The type of fructan and its distribution in the plant is related to the plant species, their developmental stage, and the environmental conditions (Kiss & Forgo, 2011).

At the industrial level, the inulin is used as a bioactive ingredient (Afoakwah et al., 2015; Ahmed, Thomas, & Khashawi, 2019) is mainly obtained from different parts of various plants belonging to the Asteraceae family. Some examples include chicory roots (*Cichorium intybus* L.), which present an inulin content of 11-20 g/100 g fresh weight (Barkhatova, Nazarenko, Kozhukhova, & Khripko, 2015; Shoaib et al., 2016), dahlia tubers (*Dahlia pinnata Cav.)* with an inulin content of 10-12 g/100 g fresh weight (Diederichsen, 2010; Shoaib, 2016), and Jerusalem Artichoke (*Helianthus tuberosus* L.) tubers (JAT) containing 10-22 g of inulin /100 g fresh weight (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2016; Barkhatova, Nazarenko, Kozhukhova, & Khripko, 2015; Gupta & Chaturvedi, 2020; Shoaib et al., 2016). The addition of inulin and FOS in food products is a practice that is allowed since they are officially recognized as natural and GRAS (generally regarded as safe) food ingredients (Gupta, Navdeep, & Kaur, 2003). Inulin from chicory roots or dahlia tubers are available as a food ingredient in the market and these products may differ in purity, DP, and free sugar content (Kelly, 2008), though different technological properties as well as biological activity can be obtained.

Inulin and FOS are classified as soluble dietary fibre and for nutrition labeling purposes they are included in the total dietary fibre content in combination with dietary fibre components from different sources (Susilowati, Aspiyanto, & Ghozali, 2017). As a dietary fibre, the recommended daily intake value for inulin and FOS is in the range of 1-15 g/day (Bonnema, Kolberg, Thomas, & Slavin, 2010; Judprasong, Tanjor, Puwastien, & Sungpuag, 2011; Khuenpet, Fukuoka, Jittanit, & Sirisansaneeyakul, 2017; Ripoll, Flourié, Megnien, Hermand, & Janssens, 2010). It should be considered that high doses of dietary fibre consumption may lead to gut discomfort or flatulence due to the gas formation as a result of microbial fermentation at the intestinal level (Hiel et al., 2019). The chain length may contribute to determining the fructan-type dietary fibre daily intake tolerance; in this sense, as informed by Turner and Lupton (2011), inulin is better tolerated than FOS.

In the optimization of the different inulin applications, a good understanding of the physio-chemical properties of inulin polymers is required. Many studies have been conducted about inulin over the last decades, but the information published is highly fragmented given the diversity of applications.

The present review focus on the methods employed to obtain inulin type-fructans from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers and the health-promoting properties attributed to these compounds. A compilation of data about the technological applications and biological activity of inulin from Jerusalem artichoke in different food matrices is also included.

2. Jerusalem artichoke

Jerusalem artichoke (JA) belongs to the Asteraceae family and is an annual plant native from North America that has emerged as an alternative source of fructans like inulin (Lv et al. 2019; Tanjor, Judprasong, Chaito, & Jogloy, 2012). So far reports about JA indicates that it is not only cultivated in North America (USA and Canada), but also in Northern Europe, China, Korea, Australia, Thailand, Yugoslavia, Austria, Hungary, Slovenia, South Africa, and New Zealand, among other countries and its tubers have become increasingly popular in many cooking recipes around the world (Bach, Jensen, Clausen, Bertram, & Edelenbos, 2013).

A total of 20 varieties of JA have been described by Berenji and Sikora (2001), while different authors characterized other cultivars from specific geographic areas (Danilčenko, Jariene, Slepetiene, Sawicka, & Zaldariene, 2017; Kocsis, Liebhard, & Praznik, 2007; Krivorotova & Sereikaite, 2014; Rébora, 2008). JA can grow in different regions without the addition of fertilizers, organic matter, or pesticides (De Santis & Frangipane, 2018). The high yield of tubers and its capacity to adapt and grow in different agroecological conditions, have contributed to expanding this crop all over the world (Pimsaen et al., 2010). Many studies have evaluated the potential of JA under different stresses such as drought (Puangbut, Jogloy, & Vorasoot, 2017), waterlogging (Yan, Zhao, Cui, Han, & Wen, 2018), and salinity (Zou et al., 2020).

The tubers are irregularly spherical or spindle-shaped and vary in color from pale brown to white, red, or purple (Long, Chi, Liu, Li, & Liu, 2009). The average weight of tubers varies from 10 to 100 g (usually 30-80 g) depending on the cultivar and growing region; however, under favorable culture conditions, the tubers can reach a weight of 500 g (Dzantieva, Tsugkieva, & Tsugkiev, 2006). The fresh tuber production may vary from 55 to 891 g/plant (Hanci et al., 2020). Generally, differences among cultivars, harvest periods, production conditions, postharvest storage, and processing methods result in variations in the fructan composition of JA tubers (Qiu et al., 2018). The fresh JAT typical composition consists of water (75-80% w/w), and a total carbohydrates content that represents up to 22 % of the weight of fresh tubers, with 70 to 90% of them being inulin (Abou-Arab, Talaat, & Abu-Salem, 2011; Barkhatova, Nazarenko, Kozhukhova, & Khripko, 2015; Puttha et al., 2012). Soluble carbohydrates, besides inulin, are its derivatives FOS, reducing sugars: fructose (Fru), glucose (Glc), and sucrose (Suc). Other minority components of JAT (expressed as %w/w) are proteins (2-3), minerals (1-2), and lipids (0.2-0.4).

Regarding the biosynthesis of inulin from sucrose in JAT, it has been well established that the two key enzymes involved in the synthesis are sucrose:sucrose 1-fructosyl transferase (1-SST) and fructan:fructan 1-fructosyl transferase (1-FFT) (Van der Meer et al., 1998). The 1-SST is the enzyme responsible for initiating inulin synthesis that produces 1-kestose, and then the 1-FFT leads to higher inulin polymers. By contrast, three members of fructan 1-fructanoexohydrolases (1-FEH) catalyze the inulin degradation to Fru and Suc (Ht1-FEH I, Ht1-FEH II, and Ht1-FEH III) (Xu et al., 2015; Zhan et al., 2018). Invertase (INV) degrades Suc into Glc and Fru while Suc is synthesized by sucrose 6-phosphate phosphatase (SPS) and sucrose 6-phosphate synthase (SPP) in source leaves, and the reaction catalyzed by SPS is thought to be the limiting step in Suc synthesis (Winter & Huber, 2000). Then, Suc is transported from the leaves through the phloem to sink tissues such as tubers and is then reversibly hydrolyzed by Sucrose synthase (SS) or INV into (UDP-) Glc and Fru (Ruan, 2014). These products of sucrose cleavage are then available for many metabolic pathways, such as energy production, primary metabolites production, and the synthesis of complex carbohydrates.

The total sugar content of the aerial portion of the plant increases to a maximum value and then decreases progressively concomitant with a period of rapid tuber development. Overall, sugar allocation declined throughout the production cycle, with a corresponding increase in allocation to the tubers and stolons (Somda, McLaurin, & Kays, 1999). Tubers expand and reach maturity by accumulating Suc which is employed as initial substrate by the 1-SST and together with 1-FFT inulin is synthetized as carbon source reserve. After dormancy, the tubers start to germinate by activating the 1-FEHs, which play critical roles in inulin hydrolysis after tuber germination (Xu et al., 2015). Thus, extracts of growing tubers contain free Glc, a product of 1-SST action, whereas Glc content decreased to low levels in the mature tubers (Saengthongpinit & Sajjaanantakul, 2005). The DP, i.e. the number of fructofuranosyl units of inulin in JAT, also varies throughout the growing season, harvesting maturity, and storage time after harvest. The inulin in mature tubers can contain from 3 to 35 fructofuranosyl units, however fructans with DP between 2 and 10 constitutes the majority (Luo et al., 2018; Panchev, Delchev, Kovacheva, & Slavov, 2011). Throughout JAT storage a decrease in inulin content and mean DP occurs, due to its depolymerization (Leroy, Grongnet, Mabeau, Corre, & Baty-Julien, 2010; Rubel et al., 2014). Due to the JAT susceptibility to rot, they must be harvested and processed as soon as possible to avoid inulin degradation; among the commonly used storage alternatives tubers can be left in the soil for overwintering (Krivorotova & Sereikaite, 2014; Saengthongpinit & Sajjaanantakul, 2005). Different studies about the growth and phenology of JA reported that during the post-rainy season, the reproductive and tuber development stages occurred faster than in the early-rainy season (Paungbut et al., 2015). As the fructan DP varies during the plants´ growth stage, early-harvested tubers present a higher amount of sugar fractions with a high DP, which offers more industrial value than late-harvested tubers or those after storage (Schorr-Galindo & Guiraud, 1997). The optimum harvesting stage based on the quality and quantity of tubers harvested in different geographical areas has been analyzed by various authors, suggesting that after 16–18 weeks they accumulate higher DP compounds than 20 weeks mature tubers (Saengthongpinit & Sajjaanantakul, 2005). Liu et al. (2015) showed that factors such as genotype, agroecological conditions and their interactions strongly influenced total soluble sugar contents in JA. Early harvested varieties (Bella and Bianka) and early middle varieties (Topstar and Gigant) harvested

22–25 weeks after plantation presented high content of water soluble carbohydrates (60–65 g/100 g dry mass). A similar amount of water-soluble carbohydrates (55–60 g/ 100g dry mass) was obtained in late varieties (Waldspindel, Violet de Rennes, Rote Zonenkugel) when harvested 29–33 weeks after planting (Kocsis, Liebhard, & Praznik, 2007). Also, Černiauskienė et al. (2018) observed that the differences in inulin content in JAT were related to the harvest time and their variety. These authors found larger amounts of inulin during early spring rather than during autumn. According to Taper and Roberfroid (2002) the mature tubers of JA contained approximately 11.7 g inulin /100 g fresh weight and 6.3 g sugar/100 g fresh weight. Li et al. (2015) suggested that before the blossoming, the DP of inulin increased rapidly and then decreased gradually at a lower speed. These authors showed that the inulin content could reach a maximum of 12.21 g/100 g fresh weight and simultaneously, the maximum inulin DP could reach a value of 19. Other studies showed that the content of inulin and the nutritional value of JAT decline markedly when the growing season begins, mainly due to its conversion to sucrose and the formation of inulin with a lower DP (Poulsen et al., 2012). As most studies suggest, tubers harvested during autumn would be the best option for minimizing sucrose levels (Krivorotova & Sereikaite, 2014). A distinctive impact on the maturing process and frost period alterations have also been reported for JA of different cultivars, which resulted in modification of inulin and sugar contents (Kocsis, Liebhard, & Praznik, 2007). In line with these observations, Danilčenko et al. (2017) proposed different harvest times for diverse cultivars, considering the products to be obtained from the tubers. For instance, they showed that autumn harvested tubers of the cultivar Sauliai were suitable as a source of inulin and FOS. In contrast, the spring-harvested tubers of the cultivars Sauliai, Rubik, and Albik were more appropriate for obtaining dry products because of their highest contents of dry matter, total phenolics, and carbohydrates. The main factors related to the JA inulin content and inulin DP are summarized in Figure 1.

Inulin application in food and beverage industries has been increasing both in countries where this ingredient is produced as well as in those countries that must import it, which impacts increasing the costs. The JA offers competitive advantages and is also an economically profitable crop representing a rich source of inulin, that can help to fulfill de increasing demand for this ingredient. The JA powder production as well as their derivative products such as inulin in Europe have stablished a large industry. Currently, there are three worldwide companies that produce JA powder, located in Belgium and the Netherlands, accounting for 98.8% of the world's annual output (Tian & Lui, 2020). Also, nine Chinese companies produce JA flour and related products (Ding, Dong, & Tan, 2006; Kayshev, Lukin, & Seryogin,2018), however as there is a big gap compared with the similar European products, they also import them. Other countries such as Russia and Thailand also import JA powder and related products for the fortification of various kinds of commercial foods such as beverages, bakery products, dairy products, confectionery, and baby food (Parker, 2013; Chaito et al., 2016; Termrittikul, Jittanit, & Sirisansaneeyakul, 2018; Kayshev, Lukin, & Seryogin,2018).

3. Jerusalem artichoke inulin extraction and purification methods

The process of inulin production from JAT has been developed and can be applied in the food industry (Khuenpet, Fukuoka, Jittanit, & Sirisansaneeyakul, 2017). The inulin extraction from JAT represents a critical step to obtain this compound purified to be employed as a bioactive ingredient in food products. The development of experimental methods for extraction, analytical quantification, and determination of the DP of inulin is of great importance for the characterization of the technological and bioactive properties of this compound. Thus, inulin can be extracted from JAT through simple solid:liquid extraction employing hot water as solvent (Figure 2). The raw material for inulin extraction from JAT could be either dried or fresh JAT followed by a milling step to increase the specific surface area of solid particles and consequently the inulin extraction efficiency. It is worth mentioning that drying tubers is necessary if the processing capacity is not enough to handle all the fresh JAT in its harvesting season. Moreover, if the JAT is sufficiently dried, it can be stored and used for inulin powder production later.

Processing fresh JAT (78 % w/w humidity) represents lower costs as long as the processing facilities of the factory are compatible with the supplied amount of the fresh JAT.

The most popular method to obtain inulin from JAT is solid-liquid extraction using hot water, as solvent. This method leads to a JAT extract that contains inulin and FOS as the main carbohydrates, a low proportion of pectins, a low percentage of proteins, and a minimal mineral content. The key factors that affect the yield of inulin extraction include temperature, extraction time, and solid to solvent ratio (Abou-Arab, Talaat, & Abu-Salem, 2011; Apolinário et al., 2014; Paseephol, Small, & Sherkat, 2007; Rubel et al., 2014, 2018; Saengkanuk, Nuchadomrong, Jogloy, Patanothai, & Srijaranai, 2011; Toneli, Park, Murr, & Martinelli, 2008). Also, many investigations have included different steps to increase the extraction efficiency such as blanching, peeling, chopping, crushing, drying, ohmic heating, direct and indirect ultrasound assistance, and high pressure (Table 1). Takeuchi and Nagashima (2011) found that blanching at 60 °C or higher could inactivate inulinase, which causes inulin degradation in JAT during storage, so a blanching process may be advisable to apply in order to prevent inulin hydrolysis. Wang and Sastry (2002) and Lebovka et al. (2005 a, b) stated that ohmic heating could induce electropermeabilization of the cell membranes. Vorobiev and Lebovka (2008) described that when the electrical current passes through the biological tissue, both temperature rise and membrane damage occur leading to the diffusion of solutes inside the cellular structure. Usually, the pH during inulin water extraction is uncontrolled and naturally remains around 6.8-7.0 (Rubel et al., 2018). Moreover, Noori et al. (2014) reported that the highest extraction rate was obtained at pH 7, while acidic or alkaline media (pH 3 or 11, respectively) were less efficient as compared to the neutral extraction, since in an environment with $pH<4$, inulin hydrolysis proceeds quite intensely, increasing the reducing sugars content. Böhm et al. (2005), Glibowski and Bukwska (2011), and Luo et al. (2011) pointed out that inulin has high stability when $pH \geq 5$, even at high processing temperatures, evidenced by the fact that there are no significant changes in the content of reducing sugars, in extracts obtained at pH between 5-12. Temperatures between 30 and 90 °C and time between 20 and

90 min were generally used for inulin extraction. Usually, the increase of extraction temperature, time and solvent proportion lead to the rise of the inulin extraction yield; nonetheless, the energy and time consumption and the solvent cost must be considered (Paseephol, Small, & Sherkat, 2007). During the whole process however, the degradation of inulin and the formation of a dark brown color must be avoided (De Leenheer, 2007). According to Bach et al. (2013), JAT turns gray after boiling due to after-cooking darkening reactions between iron and phenolic acids. Non-enzymatic browning reactions, such as the Maillard reaction, could be another reason for the darker color of the extracts.

Basically, the purification of the inulin extract can be reduced to two steps (Figure 2). In the first one (precipitation/clarification), the colloids and floating contaminants are coagulated by liming and carbonation at high pH, then precipitated and filtered. Alternatively, inulin can be precipitated with solvents such as ethanol, isopropanol, acetone, or acetonitrile (Abozed, Abdelrashid, El-kalyoubi, & Hamad, 2009). It is possible to separate fructans into fractions according to the DP, for example, by using different ethanol concentrations. Evdokimova et al. (2021) reported that fructan fractions from a JA water extract, precipitated with ethanol 20% presented higher DP than with ethanol 80%. The second step (refining) consists on the demineralization (or deionization) and decoloration of the clarified extract with ion exchangers resins and activated charcoal, respectively (Barta, 1993; De Leenheer, 2007).

As mentioned before, the aqueous inulin extracts can also be purified by different methods, such as the addition of alcohols (ethanol or isopropanol), which decrease the polarity media, leading to inulin precipitation. Although the precipitation by alcohol addition is efficient and has been widely used in the laboratory, it is deemed costly and improper for industrial-scale inulin production owing to the price of alcohol and its recovery cost (Luque-Garcia & De Castro, 2003). Consequently, the most common purification process includes carbonation, deionization, and decolorization (Jirayucharoensak, Khuenpet, Jittanit, & Sirisansaneeyakul, 2018; Zhi-fu et al., 2009). During carbonation, calcium oxide (CaO) is added into the inulin extract solution at the amount of 12%-15% of JAT powder weight, and is then continuously stirred until the pH value of the solution reaches values between 11 and 12. After that, carbon dioxide (CO_2) is introduced until the pH of the solution is reduced to 6.8-7.0. After the carbonation process, the deionization and decolorization steps are conducted by column filtration systems with specific ion-exchange resins and activated carbon, respectively (Abou-Arab, Talaat, & Abu-Salem, 2011). Alternatively, deproteinization may be carried out by treatment with a sufficient amount of $Ca(OH)_2$ until reaching pH 11. Subsequently, H_3PO_4 must be added to remove the excess of $Ca(OH)_2$ until pH 8 is attained. Khuenpet et al. (2018) observed that the purification of a JAT water extract through carbonation efficiently removed impurities such as proteins, and also decreased the contents of fructose, while the proportion of sucrose in the remaining solids significantly increased. In addition, Srinameb et al. (2015) reported no significant differences in the inulin content and molecular weight profile obtained after purification by ion-exchange resin, comparing the materials before and after this purification step.

The final step for the purified inulin powder obtaining is the application of a suitable drying method such as freeze-drying or spray-drying. The spray drying process is commonly employed in the industry considering cost, versatility, time-consuming and the capacity to have a continuous process. The drying methodologies require that JAT extract be previously concentrated to obtain better process efficiencies (Jirayucharoensak, Jittanit, & Sirisansaneeyakul, 2015). The inulin powder has the advantage that it can be stored for long periods under proper conditions. However, it must be considered that, during storage, the quality of the powder is affected by environmental factors, for example, temperature, oxygen, and relative humidity (Jirayucharoensak, Khuenpet, Jittanit, & Sirisansaneeyakul, 2018; Khuenpet et al., 2015a; Rubel et al., 2018). Inulin is very hygroscopic and readily absorbs moisture from the air when placed indoors (Tian & Lui, 2020). Experimental results showed that the obtained JAT inulin powder displays physicochemical characteristics similar to the commercial inulin standards (Abou-Arab, Talaat, & Abu-Salem, 2011; Khuenpet, Jittanit, Sirisansaneeyakul, & Srichamnong, 2018; Srinameb et al., 2015).

Inulin is soluble in water and slightly soluble in alcohol. The solubility of inulin increases with temperature, and at a specific temperature, the solubility becomes constant. Inulin solutions presents a very low viscosity, and their viscosity increases with the concentration. Inulin concentrations above 30% w/v will slowly gel and form weak gels. While, at concentrations of 50% w/v, self-supporting gels are formed immediately.

According to Guo et al. (2018) the application of inulin powder obtained by oven-drying or freeze-drying conducted to differences in textural and rheological properties of food matrices. Jirayucharoensak et al. (2018) informed that the color of the inulin powder produced under a particular spray-drying condition was significantly darker, although due to the small amount used as an additive in a food product, its effect on the color was minimal. Moreover, reports showed that the total sugar contents in inulin extract from JAT were not significantly different after evaporation and spray-drying processes (Khuenpet, Jittanit, Sirisansaneeyakul, & Srichamnong, 2018).

4. Health benefits of Jerusalem artichoke

The latest evidence suggests that a focus on the quality and diversity of the diet, particularly a diet rich in fibre, may have implications in the improvement and management of various non transmissible diseases and in complications such as intestinal dysbiosis or constipation (Reynolds et al., 2019). Dietary supplementation with vegetables naturally rich in inulin-type fructans, as JAT has been shown to exert positive health effects both attributed to dietary fibre promoting effects such as reduction of plasma lipid, induction of body weight loss, improvement of insulin sensitivity and decrease risk of diabetes as well as the prebiotic activity (Gupta & Chaturvedi, 2020; Hiel et al., 2019). The recommended daily intake of inulin is 5–15 g/day and has been reported to be beneficial to human health (Khuenpet et al., 2016). The studies that address the health effects of

JAT and the derivative products, analyses either dried tubers (where the main component is inulin, with lower proportions of proteins, cellulose, hemicelluloses, vitamins, and minerals) or inulin from JAT powder, with different inulin contents according to the purification process. As suggested by Knudsen and Hessov (1995), JAT inulin is minimally hydrolyzed in the upper gastrointestinal tract obtaining high recovery in the human small intestine, showing similar features of dietary fibre. The prebiotic activity has been widely described for inulin-type fructans in JAT water extracts (Costabile et al., 2010; Gupta & Chaturvedi, 2020). *In vitro* studies have been carried out to evaluate the growth of different probiotic strains employing JAT inulin as a carbon source. In this sense, Rattanakiat et al. (2020) observed that the growth and acid production increased when the probiotic strains *L. plantarum, L. acidophilus, B. longum* and *B. breve*, were incubated with JAT extract. Likewise, Ali et al. (2016) demonstrated that the growth of *Bifidobacterium bifidum* improved significantly in the presence of JAT fructans. Also, Rubel et al. (2014) reported a higher *in vitro* prebiotic score for inulin from JAT than commercial inulin from chicory roots, also evidencing the influence of the storage time of the tubers in this property. Moreover, JAT inulin is selectively employed as a carbon source by different probiotic bacteria (Iraporda, Rubel, Manrique, & Abraham, 2019). In a more recent *in vitro* study, Evdokimova et al. (2021) described a better growth of *Bifidobacterium bifidum* during the fermentation of JAT-inulin of low DP compared with JAT inulin of high DP, with values even higher than those obtained with glucose. These authors also showed a significant shift in the production of acids by bifidobacteria towards acetic acid when they were grown with JAT inulin, exhibiting a higher acid production during the fermentation of high DP JAT inulin. The capacity to induce changes in the gut microbial composition in animal models and in human interventions, was described not only for JAT inulin but also for the JA dried tubers (Table 2). Most of the studies showed that one of the principal effects of JAT inulin is related to the bifidogenic effect (Gupta & Chaturvedi, 2020). Many studies are also in agreement with the fact that the counts of *Bifidobacteria* as well as beneficial species in human faeces, were higher after the consumption of JAT or even incorporated both in snack bars or beverages (Kleessen et al., 2007; Ramnani et al., 2010). In line with these results, it was reported that a higher consumption of inulin-type fructan-rich vegetables, including JAT, produced beneficial modifications of the gut microbial composition and function (Hiel et al., 2019). Lee and Kang (2009) demonstrated that a diet that included JAT inulin effectively reduced weight and body fat and obesity-related body indicators.

The anti-diabetic effect is another of the most described beneficial properties of JAT. Wang et al. (2016) reported that the treatment of streptozotocin-induced diabetic rats with JAT has positive effects in the relief of symptoms of diabetes by repairing the liver damage caused by streptozotocin and also modulate glucose metabolism through the inhibition of α-glucosidase activity. Moreover, the authors observed an increase in the inhibitory effect of JAT on α-glucosidase activity by fermentation with *L. plantarum*. Therefore, the fermented JAT also showed a significant anti-hyperglycemia effect in db/db mice by increasing insulin level, decreasing insulin resistance, and delaying the absorption of carbohydrates. Results described by Yang et al. (2012) showed that JAT consumption in combination with soybeans fermented with *Bacillus* spp. improved insulin sensitivity, potentiated glucose-stimulated insulin secretion and enhanced β-cell function in the pancreas. A transcriptome analysis reported by Chang et al. (2014) showed the preventive effect of a diet supplemented with JAT (10%) in the development of type 2 diabetes and non-alcoholic fatty liver disease in rats, by reducing the expression of genes malic enzyme 1 (Me1), decorin (Dcn), and nicotinamide phosphoribosyltransferase (Nampt), which were increased by fructose feeding treatment. Additionally, the JAT supplementation decreased hepatic triacylglycerol accumulation and steatosis. The biological effects of JAT on glycemic response in diabetic rats was also reported by Zaky et al. (2009). These authors showed that consuming a diet containing JAT, reduced serum glucose levels, total cholesterol, LDL cholesterol, and triglycerides in hyperglycemic rats; also, improvements in kidney and liver functions were described. Also, Shao et al. (2020) investigated the effect of inulin from JAT on hyperglycemia, liver-related genes, and the intestinal microbiota in a diabetic mice model. They observed that inulin treatment increased the number of Bacteroides in intestinal microbiota, and concluded that this contributed to the prevention and treatment of hyperglycemia. The anti-fibrotic effect of JAT were described by Abdel-Hamid et al. (2015). The analysis of liver enzymes activities and total bilirubin levels carried out by these authors suggested that JAT treatment presented a promising hepatoprotective effect against carbon tetrachloride (CCl_4)-induced fibrosis via modulation of apoptotic signaling and fibrogenic activity. Another beneficial health effect reported by Kang et al. (2018) is the attenuation of the atopic dermatitis symptoms in a mice model topical by the topical administration of JAT. So, JA products may be considered as a promissory therapeutic agent or supplement for skin allergic inflammatory diseases. Antioxidant activity of JAT inulin against linoleic acid oxidation was also informed by Li, Gunenc & Hosseinian (2020) using a liposome model by delaying or inhibiting the production of conjugated dienes, and they attributed this effect to the remaining phenolics compounds co-extracted with inulin. Also, Lee et al. (2014) reported that JAT infusions presented high content of phenolic compounds with antioxidant activity.

5. Technological applications of Jerusalem artichoke inulin in food products

In recent years the functional food market has experimented with one of the most significant growths in response to consumers' demands for better life quality. Inulin is a bioactive compound of relevance and interest not only for its beneficial health properties but also because it provides technological advantages for the development of new products. The physicochemical and functional properties of inulin are related to the DP and the presence of branches. So, the inulin DP has a significant influence on its industrial application as a food ingredient. Short-chain inulin (DPn<10) has been employed mainly as an alternative low-calorie sweetener and to improve mouthfeel because its properties are closely related to those of other sugars. Since long-chain inulin (DPn>23) is less soluble, and leads to more viscous and thermostable suspensions, it is commonly employed for the development of food products with specific rheological and sensory properties, such as fat replacer in low-fat or reduced-fat products (Özer, 2019). The addition of long-chain inulin to food products may improve organoleptic and rheological characteristics such as taste and texture, enhance the stability of foams, emulsions, and the mouthfeel of many types of matrices. Numerous studies showed that either JAT or JAT inulin can be successfully used as bioactive ingredients in dairy products (yoghurt and cheese), bakery products (cake, biscuits, and bread), sausages and beverages (Alibekov et al., 2021; Gupta & Chaturvedi, 2020; Khuenpet, Fukuoka, Jittanit, & Sirisansaneeyakul, 2017) as listed in Table 3.

Praznik et al. (2002) applied JAT powder in wheat and rye bread. These authors informed that the loss of fructan content by hydrolysis during dough development and baking process strongly depends on the DP distribution of the fructans employed. So, the bread made with JAT powder containing low DP inulin presented higher fructose contents than the breads formulated with JAT powder containing high DP inulin; however, both bread samples showed a high quality in organoleptic evaluation. Radovanovic et al. (2014) developed wheat bread enriched with JAT powder, and reported that the product showed an optimal nutritional and caloric value and low glycemic index. JAT powder was also applied in cakes, and the results showed that this additive favored the sensory properties: aroma, texture, elasticity, porosity and softness, surface crust, appearance, color, shape, and size (Gedrovica, Kārkliņa, & Straumīte, 2010). Park et al. (2010) applied JAT powder to elaborate a traditional Korean rice cake and determined that the quality characteristics depended on the amount of the ingredient incorporated into the formulation. The JAT powder was also applied to develop cheese from cows' milk with prebiotic activity. The use of JAT powder in cheese manufacture received the highest total scores and promoted the growth of *L. acidophilus* LA-5 (Elkot, & Hussein, 2020).

Babenyshev et al. (2020) applied the JAT extract in the cottage cheese whey purification process since polysaccharides and proteins are complexed and can be separated by sedimentation, resulting in a reduction of the protein content of the whey. Afoakwah et al. (2015) concluded that the application of JAT powder as an ingredient improved the processing properties of emulsion-type sausages. Regarding dairy products, Guo et al. (2018) indicated that JAT powder enhanced the yoghurt nutritional value, sensory characteristics, and microbial counts, while also ameliorating the firmness, syneresis, and adhesiveness that was increased in low-fat yoghurt. Amal (2009) evaluated the influence of different levels of JA paste on the quality of low-fat yoghurt and showed that the highest concentration increased the syneresis and decreased firmness. However, the yoghurt samples containing the lowest concentration of JA paste presented similar quality characteristics and were not different from the control yoghurt made with whole milk. Moreover, the addition of JA paste enhanced the lactic acid bacteria growth. JAT powder was also applied in fermented milk showing high levels of lactic acid bacteria, low pH, increased viscosity and higher antioxidant activity compared to the control sample (Park, Renchinkhand, & Nam, 2019). According to Yaseen et al. (2020) the addition of JAT powder promoted the activity of *L. reuteri* and also helped to the recovery of injured cells after the end of the storage period at -18 °C for 60 days.

Hashemi et al. (2015) demonstrated that JAT inulin improved survival of *L. plantarum* LS5 in milk-based beverage during storage, while no effect of inulin on acidity, exopolysaccharide content, syneresis, and sensory analysis of samples were observed. Similar results concerning the addition of different concentrations of JAT inulin on the quality of low-fat yoghurt were described by Khudair (2018). This author reported that the yoghurt samples containing the higher JAT inulin concentration presented a high syneresis, however, the acetaldehyde content, pH value and titratable acidity were not affected. Moreover, the quality characteristics of the yoghurt containing a lower concentration of JAT inulin were similar to the control yoghurt made from whole milk. Also, textural and rheological studies developed by Kusuma et al. (2009) indicated that JAT inulin as a fat replacer in low-fat yoghurt led to similar properties of full-fat yoghurt. Also, JAT inulin showed the capacity to enhance the growth of probiotic cultures in milk as well as their viability during storage (El-Kohly & Mahrous, 2015). Semjonovs et al. (2014) demonstrated that the addition of JAT inulin to fermented cabbage juice beverages increased probiotic bacterial viability during refrigerated storage of the product. Therefore, JAT inulin resulted suitable for the use as a functional ingredient in dairy probiotic and symbiotic products.

Rubel et al. (2015) described that the enrichment of white bread with JAT inulin disrupted the starch–gluten matrix due to fibre replacement of flour, producing an increase in the crumb hardness and chewiness. Also, the breads samples with JAT inulin were significantly darker, flatter, and more humid; however, no significant differences were reported in sensory attributes with respect to the control samples. In contrast, the fortification of different commercial products such as instant cereal drink, ready mixed soya powder, rice porridge, and chocolate malt mixed beverage with JAT inulin, reduced the sensory scores of these products (Khuenpet et al., 2015b). However, a biscuit formulation including JAT powder as a substitute for wheat flour presented high rate of acceptability, resulting in a healthier alternative with lower energy content than traditional wheat flour-based biscuits (Diaz et al., 2019). Alibekov et al. (2021) developed cottage cheese fortified with JA inulin that presented physicochemical properties that met the standard requirements for cottage cheese, with a milky-white color, a pleasant uniform consistency, without odour, and a mild taste.

Moreover, Radovanovic et al. (2015) informed that products based on buckwheat and JAT processed using extrusion technology presented desirable sensory properties for their consumption either directly as breakfast cereals, snack products, or milled to be used as an additive in other food products. Li et al. (2020) and Salijanova et al. (2020) described that the addition of JAT inulin extract in water/oil emulsion gels improved emulsifying and stabilizing capacities, texture as well as retarded lipid oxidation. So, all the information exposed in this section makes evident the versatility of JAT or derivate products including JAT inulin in food products formulations.

6. Conclusion

Jerusalem artichoke is a promising natural source of inulin emerging in numerous countries as an alternative to the traditional ones and contributes to the promotion of the development of an undervalued crop. The literature is scarce regarding the interaction between the different varieties of Jerusalem artichoke with the environmental factors, growing conditions and harvest time, and consequently with the tuber yields and the physicochemical characteristics of the inulin-type fructans obtained from their tubers. The impact of these variables, and those related to storage conditions, and the methods used for extraction and purification of inulin-type fructans on the physicochemical characteristics of the product have not been deeply studied.

From all the information previously exposed, it is evident that both JAT powder and JAT constitute highly versatile food ingredients that allow a series of applications that are still poorly explored. Since they are compounds that can exert effects on technological, sensory, nutritional, and functional health aspects, they represent an attractive resource in the food industry due to their excellent cost-benefit ratio. So, this work compiles information about JAT and the inulin extracted from them, useful as a basis for future research.

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865 Tables

866 **Table 1:** Summary of the optimal experimental conditions for inulin water extraction from Jerusalem artichoke tubers.

Raw material and pretreatment	Temperature (°C)	Time (min)	Solid:Solvent (w/v)	pH	Complementary treatment	Inulin yield (%)	Total carbohydrates (inulin) (%)	DPn	Reference
Dried tubers, grounded	76	90	1:16	Natural	Stirring	94.2	85.6 (NS)	NS	Rubel et al., 2018
Dried tubers, milled	85	30	1:38	NS	Ohmic heating	17.59	83.45 (52.52)	NS	Termrittiku et al., 2018
khuenpetDried tubers, blanched, milled	85	30	1:35	NS	Ohmic heating	14.53- 17.29	88.14 (77.29)	> 2	Khuenpet et al., 2017
Dried tubers, sliced	30-35	60	1:2	NS	Vibration	90-96	NS	2-35	Barkhatova et al., 2015
Dried tuber, blanched, grounded	90	40	1:15	NS	Two times extractions	NS	NS	12-18	Li et al., 2015
Dried tubers, milled	80	20	NS	NS	High pressure	92.5	NS	3-20	Srinameb et al., 2015
Fresh tubers	70	60	1:10	7	Ultrasound assistance	99.5	NS	NS	Noori, 2014
Dried tubers, blanched	85	60	1:20	Natural	NS	68.71	96.79 (NS)	NS	Abozed et al., 2009
Dried tubers, peelled, milled	76	20	1:10	Natural	Indirect sonication	83.6	NS	NS	Lingyun et al., 2007

867 DPn: Average polymerization degree. NS: Not specified.

Table 2: Summary of studies in animal models and human interventions describing the health benefits of Jerusalem artichoke tubers and the inulin obtained from them.

Jerusalem artichoke	Model	Dosage and time of administration	Main effects	Reference
Tubers powder	Laying hens performance, egg quality and cholesterol content	Diets with 5 or 10 % w/w JAT powder – 16 wk	↑ feed efficiency and egg quality	Yildiz et al., 2006
Tubers powder	Alloxan-induced diabetic rats	Diets with 5, 10, or 15 % w/w JAT powder – 5 wk	↓Serum glucose levels, TG, TC and LDL cholesterol Improvement in liver and kidney functions	Zaky, 2009
Tubers	Diet-induced obese rats	High-fat diet with 10% JAT– 5 wk	Antiobesity: ↓ Total adipose tissue weight, adipocyte size of epididymis Antidiabetic effects: ↓ Serum glucose level	Cho et al., 2010
Inulin	Alloxan-induced diabetic rats	Basal diets with 10 or 15% w/w JAT inulin – 4 wk	↓ Blood glucose levels ↓ TC, triglyceride and total lipids. ↑ HDL cholesterol level and ↓LDL cholesterol and VLDL cholesterol levels	Gaafar et al., 2010
Inulin	Pancreatectomized diabetic rats	Diet with 5 %w/w JAT inulin – 8 wk	↑ glucose tolerance Reversing insulin resistance and enhancing β-cell function	Yang et al., 2012
Inulin	Streptomized-induced diabetic rats	Daily gavage with 1 g/kg of JAT inulin – 10 wk	↓ Serum glucose levels ↓ Serum levels of triglycerides and TC. Improved lipid profiles.	Kim & Han, 2013
Tuber powder	Healthy rats	Basal diet with JAT powder 20, 40, or 60 g/kg – 12 wk	Improved intestinal morphometry Enhanced cell-mediated immunity ↓ Intestinal pH and ammonia concentrations. ↑ Lactate and total SCFA	Samal et al., 2014
Inulin	Alloxan-induced diabetic mice	Yoghurt containing a probiotic and 2.5 or 5 % v/v JAT inulin– 4 wk	↓ Blood glucose, cholesterol levels and total lipids	El-Kholy & Mahrous 2015
Inulin	Fish (male Nile tilapia)	Basal diet with 5 or 10 g/kg JAT inulin – 8 wk	Beneficial effects on growth performance and health status	Tiengtam et al., 2015
Tuber	*Pangasus bocourti* fingerlings (average weight 3.57 g/ fish)	Diet with 5 to 160 g JAT / kg combined with *L. plantarum.* Hand-fed *ad libitum* – 12 wk	Stimulated growth, immunity and disease resistance of *Pangasius bocourti*	Van Doan et al., 2015
Tuber powder	Castrated male piglets	Cereal based diet 2 or 4 % JAT powder - 40 days	↑ *Bifidobacterium* spp. populations in the proximal and distal colon ↓ Proteolytic fermentation and activity of detrimental bacterial	Barszcz et al., 2016
Tuber	High-fat diet induced hyper-glycemic/lipidemic rats	High-fat diets 10 % JAT – 10 wk	Improved glucose tolerance and the hepatic lipid profile	Okada et al., 2017
Inulin	High-fat diet obese induced mice	Daily gavage 2.5 or 10 g inulin /kg – 4 wk	Mitigation effect on obesity ↓ TC and ↑HDL cholesterol /LDL cholesterol	Qiu-hong et al., 2017

Tuber extract	*Dermatophagoides farina*-induced atopic dermatitis mice model	Topically application of JAT extract - 4 wk	Attenuated atopic dermatitis skin symptoms ↓ Dermatitis score and inflammatory mediators	Kang et al., 2018
Tuber powder	Healthy rats	Basal diet supplemented with 2, 4 or 6% JAT powder – 12 wk	↑*Lactobacillus* spp. and *Bifidobacterium* spp. microbiota in the caecal, colonic and rectal digesta. ↑SCFA intestinal concentrations Better apparent absorption of Ca and P	Samal et al., 2017
Inulin	High-fat diet hyperlipidemic induced mice	High-fat diet, daily intragastric administration of 2.5, 5 or 10 g JAT inulin/kg – 4 wk	↓ Serum lipid, hepatic TG and TC concentrations Alleviates hyperlipidemia ↑ *Bifidobacterium* spp. populations in the intestine	Yu et al., 2018
Inulin	High-fat diet induced diabetic mice	Daily intragastric administration 2.5, 5 or 10 g inulin/kg – 4 wk	↑ Number of Bacteroides in the intestine	Shao et al., 2020
Tubers	Crossbred male pigs	Basal diet with 4, 8 or 12% JAT – 13 days	↓ Proteolytic bacteria in the gastrointestinal tract ↓ Skatole levels in the adipose tissue	Okrouhlá et al., 2020
Inulin	Healthy human volunteers (age average 23.5 years old; BMI average 22.9 kg/m^2)	Snack bars with chicory inulin or JAT inulin. Dose: One snack bar/day (1 wk), two snack bars/ day (2 wk)	↑ Bifidobacterias ↓*Bacteroides*/*Prevotella* ↓*Clostridium histolyticum*/ *Clostridium lituseburense* group in frequency Slight increase in stool frequency	Kleessen et al., 2007
Inulin	Healthy human volunteers (age range 18-50 years old)	Fruit and vegetable shots with 2.5 % JAT inulin Dose: 2 shots 100 mL/day - 3 wk	↑ Bifidobacteria levels in faeces ↑ *Lactobacillus/Enterococcus*	Ramnani et al., 2010
Tubers	Healthy human volunteers (age range 18–85 years old, BMI 20–25, H_2 producers)	Inulin type fructan-rich vegetables Dose: 15 g inulin-type fructan/day - 2 wk	Beneficial modifications of the gut microbiota composition and function	Hiel et al., 2019

BMI: Body-mass index. JAT: Jerusalem artichoke tubers. SCFA: Short-chain fatty acids. TC: Total cholesterol. TG: Triglycerides. Wk: weeks. ↑: increase. ↓: decrease.

Table 3: Technological application of Jerusalem artichoke.

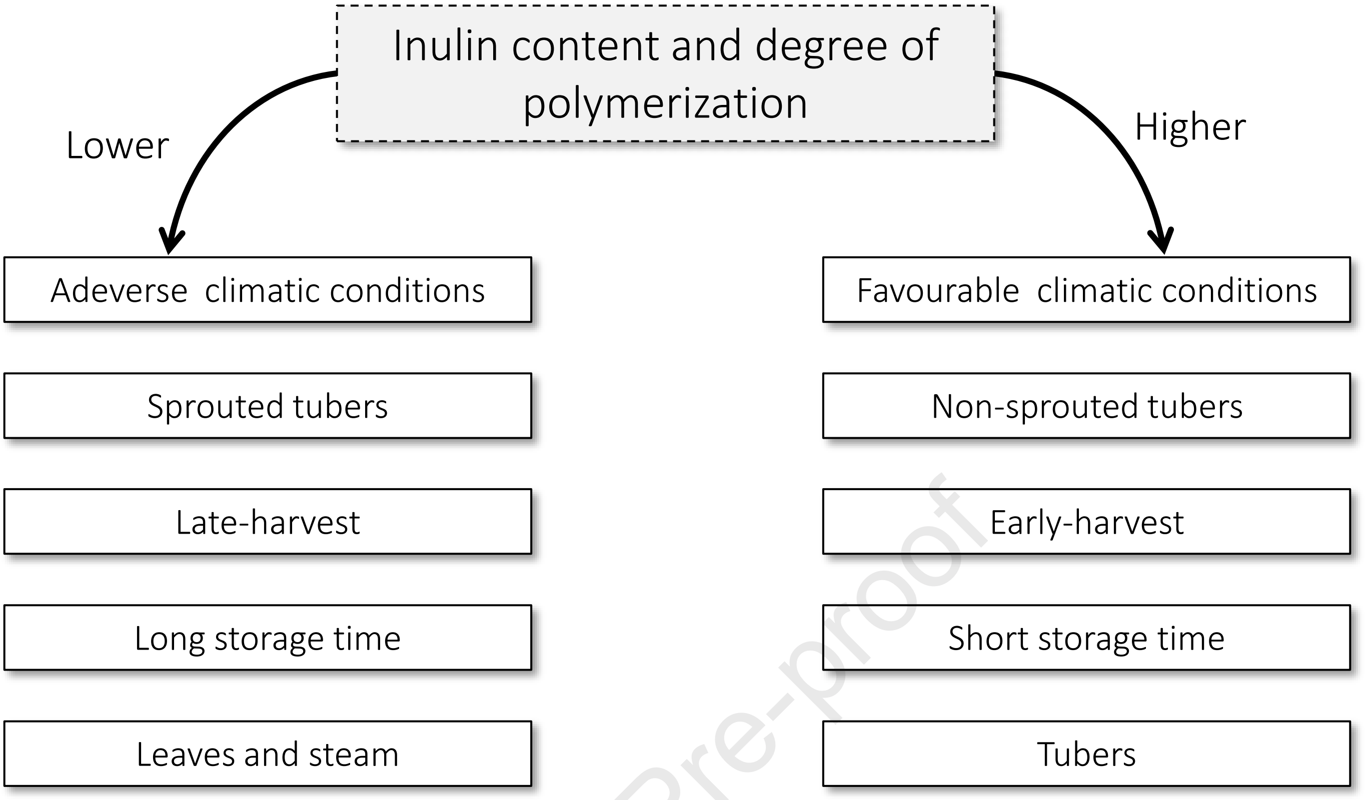
Product	JAT sample type and quantity added	Contribution	Reference
Wheat and rye bread	JAT inulin of DPn 5 or 10 – 8, 10, or 12 % w/w	High quality in organoleptic evaluation	Praznik et al., 2002
Wheat bread	JAT powder – 25 % w/w	Optimal nutritional and caloric value, low glycemic index and low glycemic load values.	Radovanovic et al., 2014
Wheat bread	JAT inulin – 2.5 or 5 % w/w	Quality attributes of the conventional bread.	Rubel et al., 2015
Cake	JAT powder – 30 % w/w	High sensory organoleptic properties.	Gedrovica et al., 2010
Biscuits	JAT flour –17, 34, 50, or 66 % w/w	Better score in the sensory panel, enhanced nutritional value and low caloric content.	Díaz et al., 2019
Yoghurt	JAT powder – 2, 4, 6 % w/v	Highest sensory score and similar textural property to the full-fat yoghurt.	Guo et al., 2017
Low-fat yoghurt	JAT inulin - NS	Quality characteristics similar to the yoghurt made from whole milk.	Khudair, 2018
Low-fat yoghurt	JAT paste – 1, 2, 3 or 4 %	Increased syneresis and decreased firmness. pH and titratable acidity were not influenced. Acceptable organoleptic properties. Promotion of the growth of lactic acid bacteria.	Amal, 2009
Low-fat yoghurt	JAT inulin - 4 % w/v	Retention of probiotics viability. Similar textural and rheological properties to full-fat yoghurt.	Kusuma et al., 2009
Cow-milk cheese	JAT powder – 1, 3 or 5 % w/w	Promotion of the growth of *L. acidophilus.* Acceptable organoleptic properties.	Elkot & Hussein, 2020
Fermented milk	JAT powder - 1, 3, 5 % w/v	Promotion of the growth of lactic acid bacteria. Increased the antioxidant and antimicrobial activity of the fermented product.	Park et al., 2019
Milk-based fermented beverage	JAT inulin – 1 or 2 % w/v	Improved survival probiotic. No effect on acidity, exopolysaccharide content and phase separation of samples. No significant effect on organoleptic properties.	Hashemi et al., 2015
Traditional Korean rice cake	JAT powder – 3, 6, or 12 % w/w	Textural properties were modified in a dose-dependent manner.	Park et al., 2010
Emulsion type-sausage	JAT powder – 3, 6, 9 % w/w	Better emulsion stability, color quality and texture. Enhanced oxidative stability, and antimicrobial properties. Extension of shelf-life.	Afoakwah et al. 2015
Commercial food products (soya powder, cereal drink, rice porridge, Chocolate/malt beverage)	JAT inulin –5 g/portion	Decreased the sensorial scores of all products	Khuenpet et al., 2016
Water/oil emulsion gels	JAT inulin – 1 % w/v	Improved the appearance and stability of emulsion gel.	Li et al., 2020
Water/oil emulsion gels	JAT inulin – 19.5 or 26.8 % w/w	Improved emulsion stability. Reduced the texture defects.	Salijanova et al., 2020

DPn: Average polymerization degree. JAT: Jerusalem artichoke tubers. NS: Not specified.

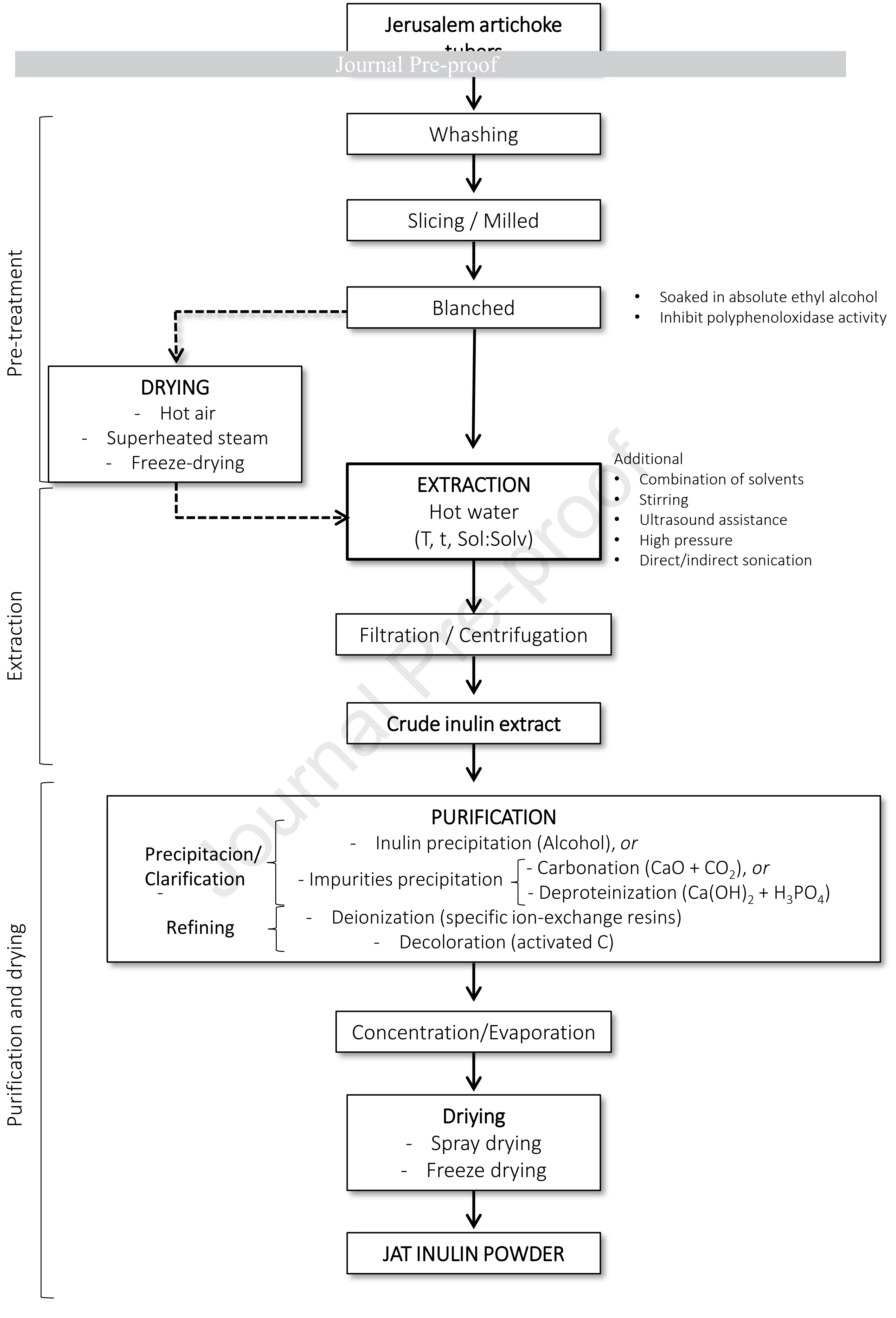
Figure captions

Figure 1: Factors that have an impact on the Jerusalem artichoke inulin content and degree of polymerization.

Figure 2: Flow diagram of the inulin production from Jerusalem artichoke tubers.



Inulin content and degree of polymerization
Lower
Higher
Adeverse climatic conditions
Favourable climatic conditions
Sprouted tubers
Non-sprouted tubers
Late-harvest
Early-harvest
Long storage time
Short storage time
Leaves and steam
Tubers


Jerusalem artichoke tubers
Whashing
Slicing / Milled
Blanched
Soaked in absolute ethyl alcohol
Inhibit polyphenoloxidase activity
DRYING
- Hot air
- Superheated steam
- Freeze-drying
EXTRACTION
Hot water
(T, t, Sol:Solv)
Additional
Combination of solvents
Stirring
Ultrasound assistance
High pressure
Direct/indirect sonication
Filtration / Centrifugation
Crude inulin extract
PURIFICATION
Precipitacion/ Clarification
- Inulin precipitation (Alcohol), or
- Impurities precipitation
- Carbonation ($CaO + CO_2$), or
- Deproteinization ($Ca(OH)_2 + H_3PO_4$)
Refining
- Deionization (specific ion-exchange resins)
- Decoloration (activated C)
Concentration/Evaporation
Driying
- Spray drying
- Freeze drying
JAT INULIN POWDER
Pre-treatment
Extraction
Purification and drying

Highlights

- Jerusalem artichoke presents a great scope for cultivation at diverse conditions
- Jerusalem artichoke tubers represents an undervalued inulin source
- Crop handling conditions determine the inulin properties as bioactive ingredient
- Inulin obtaining process defines biological activity and technological properties
- Multiple beneficial health effects are linked with inulin from Jerusalem artichoke

Conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this review article.