# PRODUCTION OF SWEETENING SYRUPS WITH FUNCTIONAL PROPERTIES

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# ABSTRACT

PRODUCTION OF SWEETENING SYRUPS
WITH FUNCTIONAL PROPERTIES

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Extraction of fructo-oligosaccharide syrups from grated jerusalem artichoke (JA) tubers was studied by water at 20-60°C by determining the yield, degree of polymerization (DP), product profile (DP of up to 6) and prebiotic effect using *Lactobacillus plantarum* on samples harvested between October and April, stored for 0-20 days. The optimum solvent to solid ratio was 4, the duration of shaking water bath extraction was 40 min and yield based on JA were 12-17%. Temperature was found to improve yield and functionality, and citric acid, at 26 mM, improved the color and darkness by 70 and 80%, respectively. Short-time (1 min) microwaving prior to extraction increased the yield by about 20%, decreased the amount of sugars with DP 1 and 2 and increased the amounts of oligosaccharides (OS) with DP 3-6, although the prebiotic effect increased only slightly; while the color and darkness of the syrup were tripled. Ultrasound-assisted-extraction (USE) gave best performance

at 3 min duration; decreased the amounts of sugars with DP 1-2, increased the amounts of OS with DP 3-6, with 18% decrease in the yield. The better functionality of USE syrups were also indicated by 2.5 times higher growth rate of L.plantarum. The application of USE at 60°C compared to 20°C almost tripled the amounts of functional sugars. In order to obtain the largest proportion of monosaccharide units as functional sugars, 10 day storage at 4°C after harvest was indicated. Ultrasonication did not affect the color but the darkness was doubled. The density and viscosity of all the syrups were practically the same.

Keywords: Fructo-oligosaccharide, Inulin, Prebiotics, Functional foods, Jerusalem artichoke, Microwaving, Ultrasonication

# ÖZ

FONKSİYONEL ÖZELLİKLERİ OLAN
TATLANDIRICI ŞURUPLARIN ÜRETİMİ

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Ekim ile Nisan arasında hasat edilmiş, 0 ila 20 gün depolanmış, rendelenmiş yer elması yumrularından frükto-oligosakkarit şuruplarının 20 ila 60°C'deki suyla özütlenmesi, verim, polimerizasyon derecesi (PD 6'ya kadar) ve prebiyotik etkileri Lactobacillus plantarum kullanımıyla belirlenerek çalışılmıştır. Çalkalayıcılı su banyosuyla özütlemede optimum çözücü/yer elması oranının 4, sürecin 40 dakika ve yer elmasını temel alan verimin ise 12 ila 17 aralığında olduğu tespit edilmiştir. Sıcaklığın verim ve fonsiyonaliteyi arttırdığı ve 26 mM sitrik asit eklenmesinin renk ve koyuluğu sırasıyla %70 ve 80 oranında düzelttiği görülmüştür. Özütleme öncesinde kısa süreli (1 dak.) mikrodalga uygulaması verimi yaklaşık %20 arttırmakta, PD 1 ve 2 olan şeker miktarını azaltmakta ve PD 3 ila 6 aralığındaki oligosakkarit (OS) miktarını arttırmakta, prebiyotik etkideki önemsiz artışa rağmen şurupların renk ve koyuluğunda üç kat artışa neden olmaktadır. Ultrason-destekli

özütleme (USE) 3 dakikalık süreçte en iyi performansı vermekte, % 18'lik verim düşüşüyle PD 1 ila 2 olan şeker miktarlarını azaltmakta, PD 3 ila 6 aralığındaki OS miktarını arttırmaktadır. USE şurupların daha fonksiyonel içeriği L.plantarum'un büyüme hızındaki 2.5 kez artışla da doğrulanmıştır. 20°C yerine 60°C'de USE uygulaması fonksiyonel şeker miktarını yaklaşık üç katına çıkarmaktadır. Monosakkarit ünitesinin en çok kısmının fonksiyonel şeker olarak eldesi için, hasat edildikten sonra 10 gün süreyle 4°C'de depolanması gerekmektedir. Ultrasonik özütleme rengi etkilememekte fakat koyuluğu iki kat arttırmaktadır. Bütün şurupların yoğunluk ve viskoziteleri hemen hemen aynı bulunmuştur.

Anahtar Kelimeler: Frükto-oligosakkarit, İnulin, Fonksiyonel gıdalar, Yer elması, Mikrodalga, Ultrasonik özütleme

To My Parents, My Husband, and to My little son ...

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| | |
|---|---|
| A | : Acidic conditions |
| AOAC | : Association of Official Analytical Chemists |
| BFM | : Bifidobacterium sp. fermented milk |
| CHD | : Coronary hearth disease |
| CLA | : Conjugated linoleic acid |
| DM | : Dry matter |
| DMA | : Dry matter Analysis |
| DP | : Degree of polymerization |
| EU | : European Union |
| F | : Fructose |
| FH | : Fructan hydrolase |
| 1-FFT | : Fructan:fructan 1-fructosyltransferase |
| 6G-FFT | : Fructan:fructan 6G-fructosyltransferase |
| FISH | : Fluorescent in situ hybridisation |
| FOS | : Fructo-oligosaccharides |
| FUFOSE | : Functional Food Science in Europe |
| G | : Glucose |
| GOS | : Galacto-oligosaccharides |
| GRAS | : Generally recognized as safe |
| GU | : Glucose Unit |
| HFCS | : High fructose corn syrup |

HPLC : High performance liquid chromatography

IBD : Inflammatory bowel disease

IFIC : International Food Information Council

IgA : Immunoglobulin A

JA : Jerusalem artichoke

MU : Monosaccharide unit

MUFAs : Monounsaturated fatty acids

NA : Non-acidic conditions

NCIMB : National Collections of Industrial an Marine Bacteria

NS : Nelson-Somogyi Analysis

NM : Not measured

PARNUTS : Foods for particular nutritional uses

ppm : parts per million

PPO : Polyphenol oxidase

PUFAs : Polyunsaturated fatty acids

RE : Reducing end

S : Sucrose

SCFAs : Short-chain fatty acids

SS : Sucrose synthase

1-SST : Sucrose:sucrose 1-fructosyltransferase

t : Tons

TOS : Transgalactosyl-oligosaccharides

UHFCS : Ultra-high fructose corn syrup

UK : United Kingdom

US : United States

USE : Ultrasound-assisted exraction

WHO : World Health Organization

WSC : Water soluble carbohydrates

$Y_{JA}$ : Yield based on 100g of jerusalem artichoke

$Y_{DM}$ : Yield based on dry matter

# CHAPTER 1

# INTRODUCTION

It is now well established that there is a clear relationship between diet and health. Although the primary role of diet is to provide enough nutrients to fulfill metabolic requirements, more recent discoveries support the hypothesis that, beyond nutrition in the conventional sense, diet may modulate various functions in the body. There has been a tremendous improvement in the knowledge of diet and genetics. Such discoveries have led to the concept of "functional food" and the development of new discipline, i.e., "functional food science". Interest in and acceptance of functional foods is gaining momentum for several reasons, including the development of new food processing, retailing, and distribution technologies; changing consumer demands and social attitudes; scientific evidence of health benefits of certain ingredients; and the search for new opportunities to add value to existing products and to increase profits.

Functional foods came and come into the market. Probiotics (live microorganisms such as lactobacilli and bifidobacteria that are added to food and that possess health-promoting properties) and prebiotics (non-digestible food ingredients that stimulate the bifidobacteria present in the colon) may be considered as the driving forces of the functional foods' market. Innovation and competition are customary in this sector. Some companies expand world-wide; others occupy strategic positions to guarantee their success. The potential of this growing market is enormous, especially when both the food and therapeutic applications of functional foods are considered.

The targets for their effects are the colonic microflora, the gastrointestinal physiology, the immune functions, the bioavailability of minerals, the metabolism of

lipids and colonic carcinogenesis. Potential health benefits include reduction of risk of colonic diseases, noninsulin-dependent diabetes, obesity, osteoporosis and cancer. The documentation of such benefits requires scientific evidence that must be evaluated. Previous assessments have concluded that, strong evidence exists for a prebiotic effect and improved bowel habit. The evidence for calcium bioavilability is promising, and positive modulation of triglyceride metabolism is undergoing preliminary evolution. Scientific research still must be done to support any "disease risk reduction claim", but sound hypothesis do already exist for designing the relevant human nutrition trials.

Probiotic products represent a strong growth area within the functional foods group and intense research efforts are under way to develop dairy products into which probiotic organisms are incorporated. Large numbers of viable microorganisms are likely to be required in the food product, which should be consumed regularly to experience the health effect.

The key focus of the functional foods market in Europe has been the development of probiotic and prebiotic dairy foods, whereas in the United States, vitamin and mineral fortification of foods in general has been the key area. As consumers become more familiar with probiotics, the demand for these products will grow. Manufacturers will respond by introducing new products that will add value to their existing portfolios. The differences in the approach to functional foods in various countries have resulted in different but related developments.

## 1.1 Functional Foods

Currently there is no universally accepted definition of functional foods. The US and the EU not only have different definitions, they have different terms to describe an

industry. While the term “functional foods” is used in EU, “nutraceuticals” is preferred in the US. Since functional food concept was originated in Japan in the late 1980s, the first definition of functional foods came from the first authority, IFIC as, foods containing “effective substances in addition to providing basic nutrition and taste” [1]. In the US, the definition is that “Nutraceuticals are naturally derived bioactive compounds, including live active cultures, that have health-promoting, disease-preventing properties, and that can be delivered in a number of different ways” [2]. Currently, the following working definition of EU Concerted Action on FUFOSE have been used: “A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved state of health and well-being and/or reduction of risk of disease” [3]. That definition describes all main features of functional foods. According to the definition features of a functional food in EU are;

- conventional or everyday food not a food supplement or a drug (no pharmaceutical product, no tablets, syrups, pills, capsules, drops or similar preparations);
- consumed as part of the normal diet;
- composed of naturally occurring components, sometimes in increased concentration or present in foods that would normally supply them;
- scientifically demonstrated positive effects on target functions beyond basic nutrition;
- provide enhancement of the state of well-being and health (“health” in the definition of the WHO means physical and social health, performance, activity and well-being) to improve the quality of the life and/or reduce of the risk of disease; and
- authorized claims.

But in US, nutraceuticals(such as supplements,herbal products and herbal medicines) areoften considered tobe theproducts produced from foods butsold inother forms(e.g., pills, powders) anddemonstrated to have physiologicalbenefits[4].Also, in

the US nutraceuticals may not be consumed as part of the normal diet, and the line between nutraceuticals and drugs often unclear.

A food can be made functional by; adding a desirable compound (antioxidants, probiotics, fiber/prebiotics, phytosterols and other functional components of plants), or removing an undesirable compound (reduction of fat, saturated and trans-fatty acids, lactose free milk for lactose malabsorbers) by technological or biotechnological means, or modifying the amount and/or bioavailability of one or more components (vitamins, calcium and other minerals, protein, conjugated linoleic acid), or any combination of these possibilities. A functional food may be functional "for all members of a population or for particular groups of the population, which might be defined, for example, by age or by genetic constitution" [5].

A functional food must also taste good and be quick and easy to prepare, be available at an acceptable price/value ratio and be considered safe. Functional foods need to be convenient and fit the image of targeted consumer group, such as offer variety, or have a 'natural' image, or be produced in an animal-friendly way.

The component that makes the food "functional" can be either an essential macronutrient if it has specific physiologic effects (such as resistant starch or omega-3- fatty acids), or an essential micronutrient if its intake is over and above the daily recommendations, or even nonnutritive value (e.g., live microorganisms or plant chemicals). Examples of food components are listed in Table 1. Among the functional components, probiotics, prebiotics and synbiotics, soluble fiber, omega-3-polyunsaturated fatty acids, conjugated linoleic acid, plant antioxidants (e.g., lycopene), and calcium are frequently mentioned.

These functional food ingredients are found in such diverse products as fermented milk and yogurt, kefirs, beverages, breakfast cereals, baby foods etc. (Table 2). Moreover, there are tablets, capsules, and powders to use as additives to foodstuffs that contain lyophilized cultures of bacteria. Lactic acid bacteria are available as pharmacopoeia preparations such as lakcid, trilac, lacidofil, and enrol 250 [6].

The global market is dominated by a large number of diversified manufacturers such as; Unilever, ConAgra, Nabisco-Kraft, Quaker, DuPont, Novartis, Pepsi, Coca-Cola, Danone, and Yakult Honcsha.

Table 1. Examples of functional components [7]

| Class/Components | Source(s) | Potential Benefit(s) |
|---|---|---|
| **Carotenoids** | | |
| Beta-carotene | carrots, various fruits | neutralizes free radicals which may damage cells; bolsters cellular antioxidant defenses |
| Lutein, Zeaxanthin | kale, collards, spinach, corn, eggs, citrus | may contribute to maintenance of healthy vision |
| Lycopene | tomatoes and processed tomato products | may contribute to maintenance of prostate health |
| **Dietary (functional & total) Fiber** | | |
| Beta glucan | oat bran, rolled oats, oat flour | may reduce the risk of CHD |
| Insoluble fiber | wheat bran | may contribute to maintenance of a healthy digestive tract |
| Soluble fiber | psylllium seed husk | may reduce the risk of CHD |
| Whole grains | cereal grains | may reduce the risk of CHD and cancer; may contribute to maintenance of healthy blood glucose levels |
| **Fatty Acids** | | |
| MUFAs | tree nuts | may reduce the risk of CHD |
| PUFAs & w-3-fatty acids | Wall/nuts, flax seeds | may contribute to maintenance of mental and visual function |
| PUFAs – w-3-fatty acids | salmon, tuna, marine and other fish oils | may reduce the risk of CHD; may contribute to maintenance of mental and visual function |
| PUFAs – CLA | beef and lamb; some cheese | may contribute to maintenance of desirable body composition and healthy immune function |
| **Flavonoids** | | |
| Anthocyanidins | berries, cherries, red grapes | bolster cellular antioxidant defenses; may contribute to maintenance of brain function |
| Flavanols – Catecins, Epicatecins, Procyanidins | tea, cocoa, chocolate, apples, grapes | may contribute to maintenance of heart health |
| Flavanones | citrus foods | neutralizes free radicals which may damage cells; bolsters cellular antioxidant defenses |
| Flavonols | onions, apples, tea, broccoli | neutralizes free radicals which may damage cells; bolsters cellular antioxidant defenses |
| Proanthocyanidins | cranberries, cocoa, apples, strawberries, grapes, wine, peanuts, cinnamon | may contribute to maintenance of urinary tract health and heart health |

Table 1 (continued)

| Class/Components | Source(s) | Potential Benefit(s) |
|---|---|---|
| **Phenols** | | |
| Caffeic acid, Ferulic acid | apples, pears, citrus fruits, some vegetables | may bolster antioxidant defenses; may contribute to maintenance of healthy vision and heart health |
| **Polyols** | | |
| Sugar alcohols – xylitol, sorbitol, mannitol, lactitol | some chewing gums and other food applications | may reduce risk of dental caries |
| **Isothiocyanates** | | |
| Sulphoraphane | cauliflower, broccoli, broccoli sprouts, cabbage, kale, horseradish | may enhance detoxification of undesirable compounds and bolster cellular antioxidant defenses |
| **Plant Stanols/Sterols** | | |
| Free Stanols/Sterols | corn, soy, wheat, wood oils, fortified foods and beverages | may reduce the risk of CHD |
| Stanol/Sterol esters | fortified table spreads, stanol ester dietary supplements | may reduce the risk of CHD |
| **Prebiotics/Probiotics** | | |
| Inulin, FOS | whole grains, onions, some fruits, garlic, honey, leeks, fortified foods and beverages | may improve gastrointestinal health; may improve calcium absorption |
| Lactobacilli, Bifidobacteria | yogurt, other dairy and non-dairy applications | may improve gastrointestinal health and systemic immunity |
| **Phytoestrogens** | | |
| Isoflavones – Daidzein, Genistein | soybeans and soy-based foods | may contribute to maintenance of bone health, healthy brain and immune function; for women maintenance of menopausal health |
| Lignans | flax, rye, some vegetables | may contribute to maintenance of hearth health and healthy immune function |
| **Soy protein** | | |
| Soy protein | soybeans and soy-based foods | may reduce risk of CHD |
| **Sulfides/Thiols** | | |
| Diallyl sulfide, Allyl methyl trisulfide | Garlic, onions, leeks, scallions | May enhance detoxification of undesirable compounds; may contribute to maintenance of heart health and healthy immune system |
| Dithiolthiones | Cruciferous vegetables | Contribute to maintenance of healthy immune function |

In 2003, nutraceuticals reached a value of $60.9 billion, globally. The US market accounted for the largest revenues in the global nutraceuticals market, followed by Europe and Asia-Pacific. Europe was the second largest market in the world for functional foods (Figure 1), with sales of US $ 9.5 billion in 2003. The leading

European country in terms of value was the UK, with sales of US $ 2.6 billion followed by Germany (US $ 2.4 billion), France (US $ 1.4 billion) and Italy (US $ 1.2 billion). Up to 2008, the market is expected to continue growing but a slower rate than in 1999-2003. By 2008, the market is forecast to reach a value of $89.8 billion, an increase of 47.6% since 2003 [8].

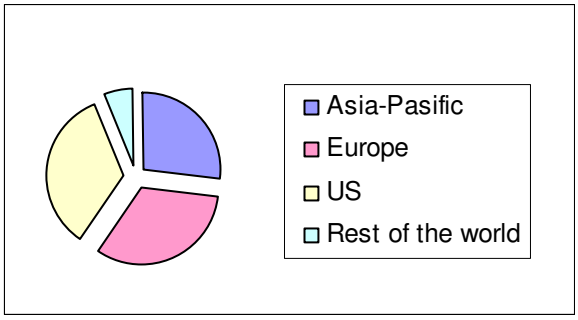


Figure 1. Global nutraceuticals market segmentation I, 2003 [8]

The leading revenue source for the global nutraceuticals market in 2003 was the dairy products sector, which accounted for 38.9% of the market's value. In value terms this sector was worth $23.7 billion. Soft drinks, and bakery and cereal products were the next largest sectors of the nutraceuticals market, accounting for 24.9% and 24.6% of the global sales respectively (Figure 2).

Table 2. Currently available functional food products [9]

| **Product** | **Trade Name** | **Functional Property** | **Company** |
|---|---|---|---|
| Yogurts | a. Activia | contains bifidus culture | Danone |
| | b. Actimel | contains L. caseii | |
| | c. Vifit | contains a live culture brand | Campina-Melkunie |
| | d. Symbalance | contains the prebiotic inulin and L. reuterii, L. acidophilus, L.casei | ToniLait AG |
| | e. Yovita | contains prebiotic fiber, Acidophilus and Bifidus cultures | Sutaş |
| | f. Babymix | contains L.rhamnosus, Bifidus sp. | |
| | g. Büyümix | Protein, vitamin, calcium enriched | |
| | h. Danino | Protein, calcium, B2, B12, D3 vitamins enriched | |
| Kefir | a. Basic Plus | | Lifeway Foods |
| | b. Kefirix | contains kefir cultures | Altınkılıç & Eker |
| Drinks and beverages | a. Bikkle | contains bifidobacterial cultures, whey minerals, xylooligosaccharides, and dietary fiber | Suntory |
| | b. Yakult | contains beneficial live bacteria | Yakult Honcsha |
| | c. LC1 | contains the L.acidophilus strain LA1 | Nestlé |
| | d. Yovita | contains prebiotic fiber, Acidophilus and Bifidus cultures | Sütaş |
| | e. Denge | omega 3 enriched | Pınar |
| | f. Lunch | soup contains inulin, oat bran, vitamins and folic acid | Otacı |
| Breakfast cereals | a. Special K | contains high amounts of fiber and folic acid | Kellogg |
| | b. All-Bran | contains high amounts of fiber and folic acid | Kellogg |
| Baby foods | a. Aptamil | contains prebiotic fiber | Milupa |
| | b. Nutrilon | contains prebiotic fiber | Nutricia |
| | c. Ceralino | contains prebiotic fiber, enriched omega 3 & 6, vitamins, iron and minerals | Hero |
| | d. Lactum | contains prebiotic fiber, enriched omega 3 & 6, vitamins, iron and minerals | Hero |
| Margarine | Benecol | Plant sterols | McNeil Consumer Health |
| | Becel | Enriched omega 3 & 6, A, D, E, B6 & B12 vitamins and folic acid | Unilever |

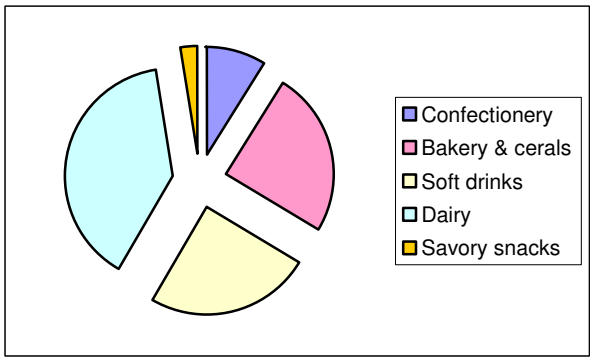


Figure 2. Global nutraceuticals market segmentation II, 2003. [8]

Danone, Doğadan, GıdaSa, Hero, Otacı, Pınar, Sütaş, and Unilever Türkiye are the companies of this sector in Turkey. Yogurt and yogurt-based drinks, baby foods, margarine are specific functional foods consumed in our country. Penetration of these foods into homes was reached 35% in 2005 with 10% increase in a year. Functional yogurt market was worth a value of 35 million YTL in 2005. It had the largest segment of the market. Now, in Turkey 1 million families have been consuming Activia [10].

Regarding functional foods, claims associated with specific food products is the preferable mean of communicating to consumers, provided these claims are true and not misleading, as well as scientifically valid, unambiguous and clear. A general definition of claim is widely accepted in the field of nutrition, as: "any representation, which states, suggests or implies that a food has certain characteristics relating to its origin, nutritional properties… or any other quality" (Codex Alimentarius, 1991). Two types of claims are specific for functional foods; the type A: enhanced function which refers to the positive consequence(s) of the interaction(s) between a food component and specific genomic, biochemical, cellular or physiologic function(s) without direct reference to any health benefit or reduction of risk of a disease, and type B: disease risk reduction that refers to the reduction of the risk of a disease by consuming a specific component or ingredient or a mixture of food component(s) or

food ingredient(s) [3]. Examples of type A are prebiotics and synbiotics, colonic food and bifidogenic factors and examples of type B include the reduction of risk of cardiovascular disease, intestinal infections, diarrhoea, constipation, osteoporosis and syndrome X (e.g., noninsulin-dependent diabetes or obesity) [11].

Although the future for functional foods appears promising, it ultimately depends on scientific evidence of their efficacy, safety, and organoleptic quality. Importantly, consumers must become aware of the beneficial health effects of functional foods.

### 1.1.1 Probiotics

The resident bacterial microflora of the human colon comprises approximately 95% of the total cells of the body and plays a key role in the host nutrition and health. In general, most gut bacteria can be divided into groups that exert detrimental effects or those that benefit the host producing beneficial compounds from carbohydrate metabolism and inhibiting the growth of harmful bacteria (Figure 3).

Two separate approaches exist to increase the number of health-promoting organisms in the gastrointestinal tract. One manner in which modulation of the gut microflora composition has been attempted is through the use of live microbial dietary additions, as probiotics. Probiotics are understood to be “living micro-organisms which have, when ingested in certain amounts, a positive effect on health beyond basal traditional inherent nutritional effects”. This definition fits well in with that of functional foods [12].

Probiotics have a long history. In 1907, Metchnikoff refined the treatment of using pure cultures of what is know called *Lactobacillus delbrueckii subsp.bulgaricus*

which, with *Streptococcus salivarius subsp.thermophilus*, is used to ferment milk in the production of traditional yogurt [13].

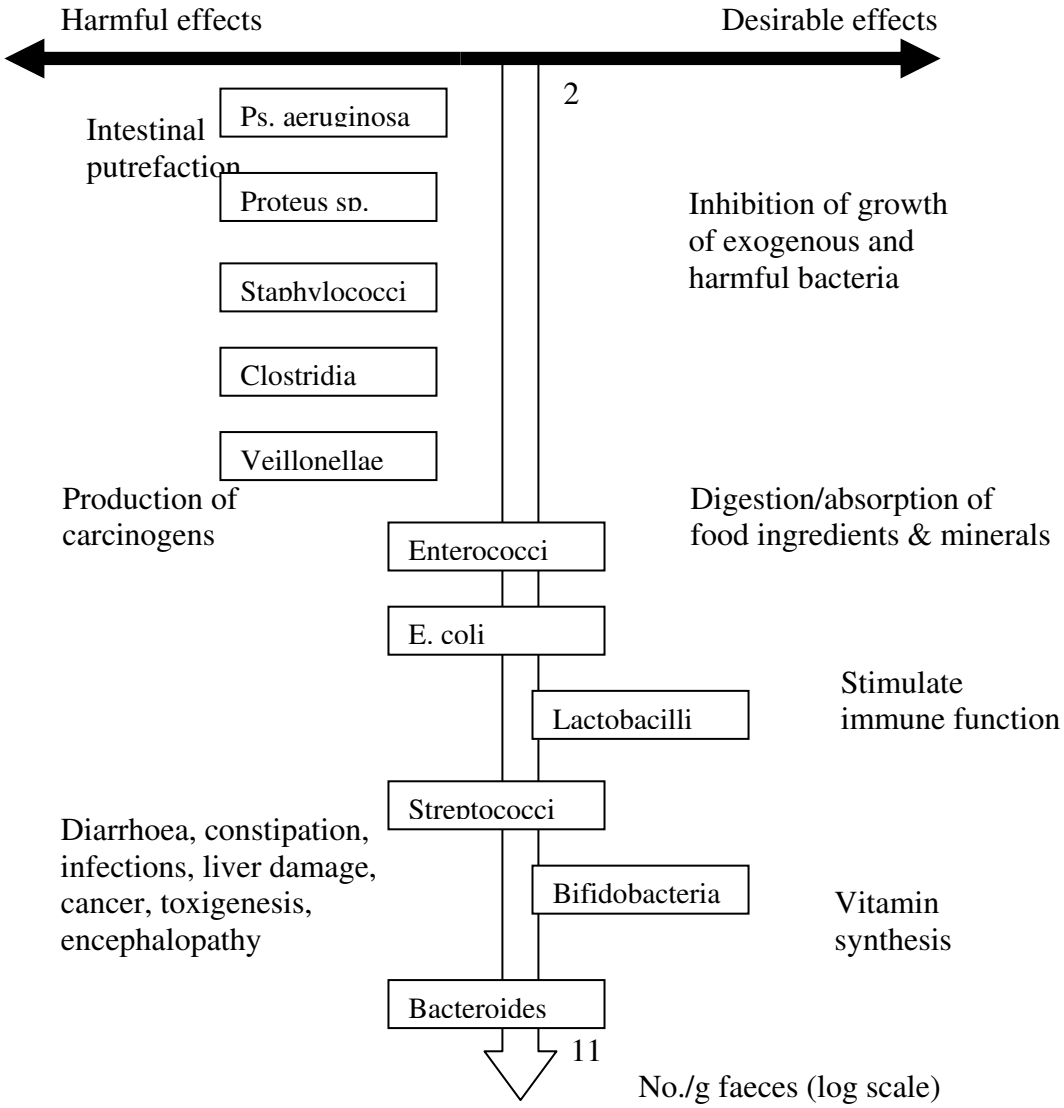


Figure 3. A simplified view of the colonic ecosystem. Bacterial groups to the left of the bar are predominantly negative in their effects on human health whilst those to the right are beneficial. Some groups are on both sides of the bar; these contain both beneficial and harmful species [12].

Microorganisms that are principally used as probiotics include two genera: Lactobacillus (naturally found in the human small intestine) and Bifidobacterium (a major organism in the human large intestine). Nonpathogenic yeasts, *Saccharomyces boulardii* and *Aspergillus spp.*, have also been used in both animal studies and

clinical trials [13, 14]. Members of the genera Lactobacillus and Bifidobacterium are most commonly given GRAS status [15]. Both genera are lactic acid bacteria, but due to technical reasons (as lactobacilli are facultative anaerobes and can tolerate exposure to oxygen during food formulation, transport and storage); most of the probiotics incorporated into food products are species of Lactobacillus. In terms of biological activity, however, Bifidobacterium species may be a preferred choice in that they generally produce more potent anti-microbial activities. As these species are obligate anaerobes, they are more difficult to incorporate into food products [12].

Common probiotics include the following: *Lactobacillus reuteri, L.johnsonii, L.gasseri, L.acidophilus, L.casei, L.reuteri, L.brevis, L.plantarum, L.rhamnosus, L.bulgaricus, L.salivarius, Bifidobacterium bifidum, B.breve, B.longum, B.adolescentis, B.animalis, B.thermophilum, B.lactis, B.infantis, Streptococcus faecium, S. thermophilus* [1, 12].

*Lactobacillus plantarum* is a gram-positive bacteria found in naturally fermented foods, vegetables, fish, and meat and also in human gastrointestinal tract [16]. It has the unique ability to "liquefy gelatin". Also, it has the ability to block receptor sites for gram-negative bacteria and so is effective as an antibiotic (e.g. plantaricin). It is an important player in antimicrobial defense and is effective against both extra- and intra-cellular pathogens. *L.plantarum* is also capable of digesting semi-digestible fibers such as those found in onions, garlic, wheat and oats. It may therefore help with digestive problems like gas and bloating. *L.plantarum* is extremely resistant to stress conditions including high temperature and concentrations of ethanol, extremes of pH and the freeze drying process that would normally kill lactic acid bacteria [17].

Additionally, *L.plantarum* is well-preserved but it seems also to preserve and increase the content of important nutrients such as omega-3 fatty acids in the food during storage [18]. Its unique ability to inhibit pathogens is also used by the food industry for food biopreservation, for example, to keep food-borne pathogens and spoilage microorganisms out of bioprocessed food, thereby dramatically increasing

the self-life of a product; e.g. tempeh, a nutritionally valuable fermentation product of partially cooked soybeans used as a meat substitute [19].

*L.plantarum* was shown to ferment fructooligosaccharides by Kaplan [20]. Selective utilization of these sugars by *L.plantarum* can improve health benefits that probiotic bacteria exert [21].

Probiotics are widely used to prepare fermented dairy products such as yogurt or freeze-dried cultures, and are used to ferment wine, vegetables (sauerkraut), sausage, and cottage cheese, and infant formulas in Europe and Japan [22]. Probiotic use may take the form of powders, tablets, capsule fillings, sachets, sprays, pills and pastes [13, 23]. In the future, they may also be found in fermented vegetables and meats [6].

Probiotic dairy foods are an important segment of the functional foods market [1]. In 1998, the European probiotic yogurt market alone was estimated to be worth a value in the region of £520 million, with the UK market reported as being the fastest growing [13]. The market for probiotics is clearly underdeveloped in the US, compared to both Europe and Japan [2]. In the regulatory arena, probiotics have perhaps a distinct advantage over other functional foods because of their cultural acceptance. In France, probiotics have had wider latitude in terms of regulatory acceptance, and in the EU a more comfortable place in both the PARNUTS and novel foods area [2].

There are four key areas of selection criteria for probiotics. These are:

- appropriateness; they should be normal inhabitants of the species targeted, and they should be non-toxic, non-pathogenic and non-inflammatory [2],
- technological suitability; they should be viable during manufacturing and also storage, they should be genetically stable and amenable [2, 23], they should have good sensory qualities [13],
- competitiveness; they should be able to adhere to the intestinal epithelium and colonize the intestine and resistance to acid and bile [24],

- performance and functionality; the bacteria should have a desirable metabolic activity; they should have an impact as antimicrobials; and they should be immunostimulatory [2, 23].

Although in some cases the mechanisms behind these effects have not been fully elucidated several health-related effects are associated with the intake of probiotics, including:

- Alleviation of lactose intolerance [24, 25]: Lactose intolerance is a problem for 70% of the world's population who have a low amount of intestinal β-galactosidase activity which digests the lactose and for whom lactose behaves like an osmotic, nondigestible carbohydrate. In clinical practice, replacement of milk by yogurt fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* or fermented dairy products decreases or suppresses the symptoms of lactose intolerance. Bifidobacteria may improve lactose digestion because these probiotics have a relatively high level of ß-galactosidase activity and are stable under normal storage conditions.
- Reduce the risk and duration of diarrhoea [26-35]: Diarrhoea occurs in up to 20% of patients who receive antibiotics, and results from microbial imbalance, due to rotavirus infection results in partial destruction of the intestinal mucosa. When a person receives anti-fungal and/or anti-bacterial therapy and the pathogenic organisms are killed off space, within the intestines and along the intestinal wall becomes available for colonization by other organisms. Taking probiotic supplements enhances the chances of these new colonies being made up of beneficial bacteria rather than more pathogenic types. Probiotics have been shown to reduce significantly the duration of diarrhoea and gut permeability caused by rotavirus. Also the gut immune defense is promoted. If the probiotic is administered early in the course of acute diarrhoea, the clinical benefit is greater.
- Prevention or reduction of the severity of IBD[36-44]: IBDs are disorders of unknown cause characterized by chronic or recurrent intestinal inflammation. They include ulcerative colitis, Crohn's disease, and pouchitis. The mechanism responsible for initiation and perpetuation of the inflammatory

process remains unknown but the main theory is that IBD may result from abnormal host responses to some members of the intestinal flora, and/or from a defective mucosal barrier.

- Reduction in the risk of colon cancer [45, 46]: Regular consumption of some probiotics may decrease the faecal levels of enzymes (e.g., ß-glucuronidase, azoreductase, nitroreductase) associated with the conversion of procarcinogens to carcinogens, mutagens, secondary bile salts which may be involved in colonic carcinogenesis, and the number of aberrant crypt foci, a marker for risk of cancer development after treatment with a chemical carcinogen. Other mechanisms may be stimulating the host's immunological defenses, and altering the pH of the colon rendering the environment less conductive to the development of cancer. Probiotics may have a role in cancer prevention by influencing microbial flora and improving nutrient bioavailability. In addition, they may have immunologic effects, may stimulate IgA response, and may effect production of cytokines.
- Immune enhancement [47-49]: Various Lactobacillus and Bifidobacteria strains per se or in foods such as yogurt have been demonstrated to enhance nonspecific immunity by increasing macrophage activation and natural killer cell activity by increasing total and specific serum IgA antibodies, to preventing the adherence, establishment and replication of several pathogens through antimicrobial mechanisms.
- Reduced intestinal infections especially *Clostridium difficile* and *Helicobacter pylori* [50-54]: Colonization of the stomach with *Helicobacter pylori* is a worldwide condition, and is initiated during childhood. Long-term colonization of *H.pylori* is clearly associated with chronic gastritis, peptic ulcers with an increased risk factor for gastric cancer in humans [55, 56]. Many lactobacilli and bifidobacterial species are able to excrete natural antibiotics, which can have a broad spectrum of activity (e.g., curvacins, nisin or bifidocin) [57-61]. Other studies suggest that probiotics can also produce metabolic products (e.g. arginine and glutamine and SCFAs) that secondarily function as protective nutrients [62].

Another proposed benefits of probiotics are prevention of atopic eczema [2, 63] and cow's milk allergy [2, 12, 13, 64, 65], allergic dermatitis [64, 66], reduction in irritable bowel syndrome [1, 67, 68], reduction in cholesterol and/or triglyceride [49, 62, 69], decreased risk of heart disease [1, 13], hormonal regulation [1, 55], increase in bioavailability of minerals, especially calcium [2, 70]. Strength of evidences for these benefits of probiotics is summarized in Tables 3 and 4.

Table 3. Strength of evidence for improvement of body functions by probiotics [70]

| **Functional effects** | **Strength of evidence** |
|---|---|
| Lactose intolerance | Strong |
| Immunostimulation | Preliminary |
| Fecal mutagenesis | Preliminary |
| Hypocholesterolemia | No effect |
| Hypolipidemia | Unkown |
| Colonic flora | Preliminary |
| Calcium bioavailability | Unkown |

Table 4. Strength of evidence for disease risk reduction by probiotics [70]

| **Disease risk reduction** | **Strength of evidence** |
|---|---|
| Diarrhea | Promising |
| Constipation | Unkown |
| Colon cancer | Preliminary |
| Osteoporosis | Unkown |
| Lipid-associated chronic disease | Probably no reduction |

When scientists better understand the mechanistic basis of probiotic functionality, they will be in a better position to understand survival/tolerance, metabolic activities, antimicrobial activity and immunostimulation.

### 1.1.2 Prebiotics

Whilst probiotics have a long history of technological development, there are some concerns over their efficacy. Probiotics should have ability to survive heat, light, moisture etc. during the time from processing to use [1]. Because probiotics do not permanently colonize the intestine, they must be taken in sufficient quantities (> 1 x $10^{10}$/d) to maintain adequate amounts in the colon [14]. Thus, a probiotic food should contain a large number of viable cells. After consumption, the bacteria must survive passage through the stomach at pH 1-3 then through the small intestine where they will be exposed to toxic bile compounds and pancreatic enzymes. Upon reaching the colon, the ingested bacteria must successfully compete with the resident bacteria. It is unlikely that all of the probiotic strains currently in commercial use can achieve this, therefore prebiotic approach is supposed to be the solution [12].

A second strategy to increasing numbers of health-promoting organisms in the gastrointestinal tract is to supply those already present in the intestine with a selective carbon and energy source.

A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one, or a limited number, of bacteria in the colon that can improve host health [11]. Probiotics mentioned above, especially bifidobacteria and lactic acid bacteria are the sole components of the colonic microflora that has been targeted. Although, both strategies share common mechanism of action (increasing beneficial bacteria in the colon) they differ in their composition and metabolism [56]. Prebiotics potentially provide some advantages over the probiotic strategy, since there is no concern about prebiotics being destroyed while in storage or en route to the intestines through the stomach acid and digestive enzymes.

Any fermentable dietary component that arrives undigested in the colon has the potential to act as a prebiotic. To date almost all prebiotics described and all those produced commercially have been carbohydrates. These range from small sugar alcohols and disaccharides, to oligosaccharides, and large polysaccharides, all with a variety of sugar compositions and glycosidic linkages. Such a diverse range of chemical structures would be expected to provide an equally diverse range of effects on the colonic microflora [71].

For a food ingredient to be classified as a prebiotic it must fulfill the following criteria [72]:

- Neither be hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract.
- Be selectively fermented by one or a limited number of potentially beneficial bacteria commensal to the colon which are stimulated to grow and/or become metabolically activated.
- Prebiotics must be able to alter the colonic microflora towards a healthier composition.
- It must preferably induce effects that are beneficial to the host health.

Examples of prebiotics:

- Disaccharides [6, 71]: Lactulose and lactitol.
- Oligosaccharides [12, 15, 71-77]: FOS, TOS, soybean oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides, lactosucrose, palatinose, polycondensates, and β-gluco-oligosaccharides produced from oat β-glucan, manno-oligosaccharides.
- Polysaccharides [14, 15, 76-80]: Inulin and resistant starch, non-starch polysaccharides.

Foods containing these compounds, prebiotic foods, are onions, asparagus, tomatoes, garlic, artichokes, honey, bananas, oatmeal, flax seeds, jerusalem artichokes, barley, whole grains, greens (dandelion greens, spinach, kale etc.), berries with seeds

(blueberries, strawberries, etc.), and legumes (lentils, black beans, chickpeas etc.) [13].

Prebiotics have already been introduced as food additives in Europe and Japan [14]. Prebiotics have been sold in Turkey are especially conducted on infant formulas like Aptamil (Milupa), Nutrilon (Nutricia), Ceralino and Lactum (Hero) all containing prebiotic fiber. Also, there are some soups, for example Lunch (Otacı) containing inulin, and dietary biscuits, e.g. Mavi-Yeşil (Ülker) containing oligofructose.

Fermentation of prebiotics to SCFAs is central to many of the proposed mechanisms for health effects provided by prebiotics (Figure 4). The profile of metabolites generated in colonic fermentation depends on the quality of the microflora and on the availability of specific substrates. The main metabolic end products of colonic fermentation are combustible gases (hydrogen, carbon dioxide, methane), biomass, lactate, succinate, ammonia, amines, phenols, indols, and SCFAs which are further used by the host organism as an energy source (namely acetate, butyrate and propionate) [14, 71, 79]. Globally, they modify the general ecology of the lumen, reducing pH and thereby controlling nonacidophilic bacteria. Moreover, these products may improve colonic absorption of calcium, magnesium, and iron.

With regard to a possible role of prebiotics in improvement of body functions and reducing the risk of diseases, the evidence is much more limited; in most cases it is either preliminary or there is no evidence at all (Table 5 & 6). The only area where the evidence can be considered promising for prebiotics is constipation. For prebiotics preliminary animal data support anticarcinogenic effects. Even though prebiotics improve calcium absorption, their positive role in reducing the risk of osteoporosis needs to be supported by more human studies. Prebiotics can not be claimed to reduce risk of cardiovascular disease.

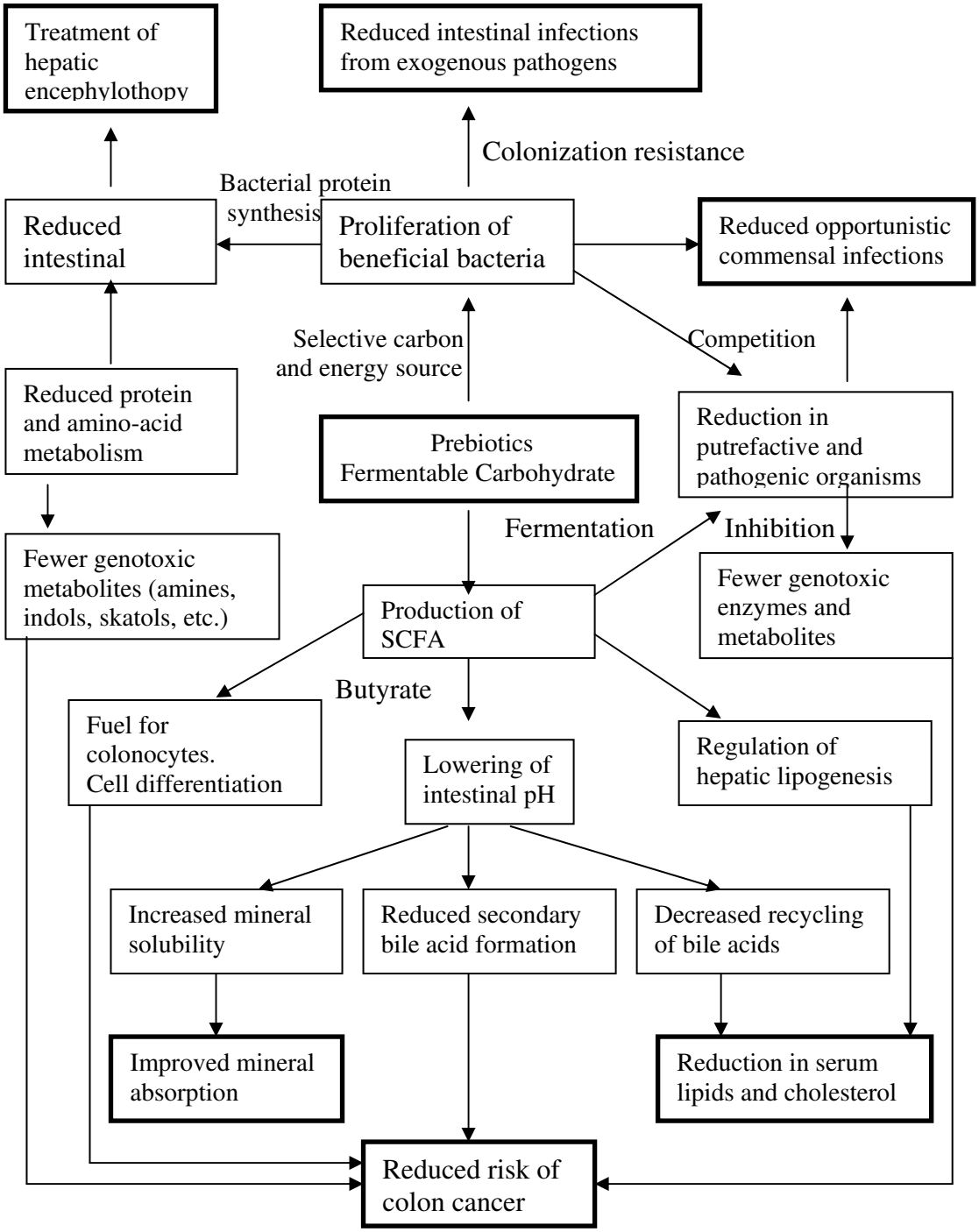


Figure 4. Proposed mechanism of prebiotic action to improve human health [71].

Table 5. Strength of evidence for improvement of body functions by prebiotics [70]

| **Functional effects** | **Strength of evidence for prebiotics** |
|---|---|
| Lactose intolerance | Unknown |
| Immunostimulation | Unknown |
| Fecal mutagenesis | Unknown |
| Hypocholesrolemia | Preliminary |
| Hypolipidemia | Promising |
| Colonic flora | Strong |
| Calcium bioavailability | Promising |

Table 6. Strength of evidence for disease risk reduction by prebiotics [70].

| **Disease risk reduction** | **Strength of evidence for prebiotics** |
|---|---|
| Diarrhea | Unknown |
| Constipation | Promising |
| Colon cancer | Preliminary |
| Osteoporosis | Unkown |
| Lipid associated chronic disease | Unkown |

### 1.1.3 Synbiotics

Synbiotics as defined by Gibson and Roberfroid (1995) are "mixtures of pro- and prebiotics" [11]. A synbiotic therefore has a beneficial effect by, firstly, supplying exogenous bacteria and, secondly, stimulating the growth of endogenous colonic bacteria. The expected benefits of synbiotics could be improved survival during passage of the probiotics through the upper gastrointestinal tract and a more efficient implantation in the host colon [6]. The probiotic will have a greater tolerance for oxygen, low pH, and temperature, and will be able to compete with other bacteria for nourishment [22]. This would then give probiotic a selective advantage over those that are indigenous [12]. Synbiotics combine the advantages of both the probiotic and the prebiotic approach. Their effects might even be additive or synergistic [6, 14, and 77]. Unfortunately they have been studied to limited extent and further work is needed to validate the concept.

Synbiotic products are generally FOS or inulin together with probiotic Bifidobacterium or Lactobacillus species have been used in yogurts. Examples of synbiotics are given in Table 7.

Table 7. Examples of commercial synbiotics.

| **Product** | **Company** | **Active Ingredients** |
|---|---|---|
| Symbalance (yogurt) | Tonilait (Switzerland) | Three Lactobacillus strains plus inulin |
| Probiotic plus FOS | Bauer (Germany) | Two Lactobacillus strains plus FOS |
| Actimel (Cholesterol control yogurt) | Danone (Belgium) | *L.acidophilus* plus FOS (from sucrose) |
| Yovita (yogurt & drinks) | Sütaş (Turkey) | Acidophilus, Bifidus & prebiotic fiber |
| Fysiq (dairy drink) | Mona (Holland) | *L.acidphilus* plus inulin |

## 1.2 Inulin and Fructo-oligosaccharides

One example of prebiotics is the inulin-type fructans; inulin and fructo-oligosaccharides. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of β (2-1) fructosyl-fructose links. A starting glucose moiety can be present, but it is not necessary. Thus the first monomer of the chain is either a β-D-glucopyranosyl or β-D-fructopyranosyl residue. When referring to the definition of inulin, both $GF_n$ (fructan molecule with a DP of n+1 and containing one terminal α-(1-2)-linked glucose) and $F_m$ (fructofuranosyl-only fructan molecule with a DP of m) compounds are considered to be included. The molecular structure of inulin compounds are shown in Figure 5. From a structural/polymeric viewpoint, (linear) inulin can be considered as a polyoxyethylene backbone to which fructose moieties are attached, as are the steps of a spiral staircase.

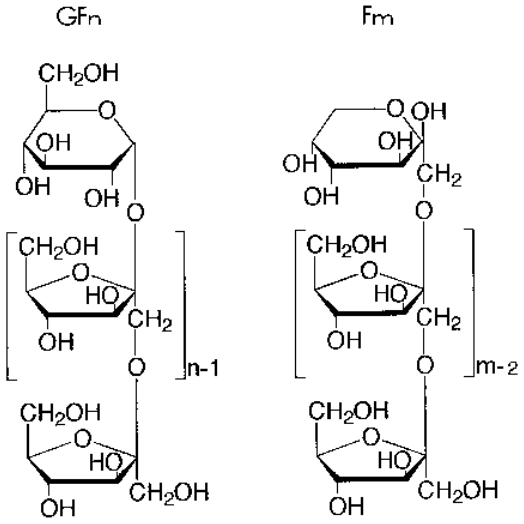


Figure 5. Inulin structure. The general formula may be depicted as $GF_n$ and $F_m$, with G being a terminal glucose unit, F representing the fructose residue and n or m characterizing the number of fructose units [81].

Rose, a German scientist, first isolated a "peculiar substance of plant origin" from a boiling water extract of *Inula helenium* in 1804, and the substance was latter called inulin by Thomson. The German plant physiologist Julius Sachs was a pioneer in fructan research and, by using only a microscope was able to detect the spherocrystals of inulin in the tubers of dahlia, *Helianthus tuberosus* and *Inula helenium* after ethanol precipitation [81].

The first reference to production of inulin from chicory being consumed by humans was made by Pedanios Dioscoride who praised the plant for its beneficial effects on the stomach, liver, and kidneys. Much later Baillarge stated that in about 1850, jerusalem artichoke pulp was added in a 50:50 ratio to flour when baking bread to provide cheap food for laborers [81].

Inulins are mainly of plant origin (Table 8), though fungal and bacterial inulin-type substances are known. Inulin-producing plant species are found in several monocotyledonous and dicotyledonous families, including Lilicaeae, Amaryllidaceae, Gramineae, and Compositae. In Liliaceae, Amaryllidaceae and Compositae, inulins

are usually stored in organs such as bulbs, tubers and tuberous roots which because of the absence of interfering components, can be easily extracted and processed to purified products. The most common plant sources are chicory (*Cichorium intybus*), jerusalem artichoke (*Helianthus tuberosus*), asparagus, dahlia, wheat, onions, banana, garlic, leek, tomatoes, and yacon [81, 82]. Especially the chicory has great potential, with the largest biomass production known, and a carbohydrate production level similar to that of sugar-beet and potato [83]. Due to its characteristics and its wide availability, chicory has been used as the source of inulin, but can not be directly consumed by humans due to its bitter taste [15].

Table 8. Inulin content (% of fresh weight) of plants that are commonly used for human nutrition [81].

| **Source** | **Edible parts** | **Dry solids content** | **Inulin content** |
|---|---|---|---|
| Onion | Bulb | 6-16 | 2-6 |
| Jerusalem artichoke | Tuber | 19-25 | 14-19 |
| Chicory | Root | 20-25 | 15-20 |
| Leek | Bulb | 15-20* | 3-10 |
| Garlic | Bulb | 40-45* | 9-16 |
| Artichoke | Leaves-heart | 14-16 | 3-10 |
| Banana | Fruit | 24-26 | 0.3-0.7 |
| Rye | Cereal | 88-90 | 0.5-1* |
| Dandelion | Leaves | 50-55* | 12-15 |
| Burdock | Root | 21-25 | 3.5-4.0 |
| Yacon | Root | 13-31 | 3-19 |
| Salsify | Root | 20-22 | 4-11 |

*Estimated value

Two enzymes responsible for inulin synthesis were isolated from plants, 1-SST (EC 2.4.1.99) and 1-FFT (EC 2.4.1.100). 1-SST synthesizes the trisaccharide 1-kestose. 1-FFT uses the 1-kestose with a higher degree of polymerization as a fructose donor and can use a variety of fructans as well as sucrose as acceptor. 1-FFT is therefore able to synthesize fructan molecules with a DP above three [84].

In Lilicaeae a different type of inulin than Compositae is present, named inulin neoseries. In that, two β (1-2) linked fructose chains are attached to the sucrose

starter unit. One chain is linked to the $C_1$ of the fructose residue (as is the case in inulin) and the other the $C_6$ of the glucose residue. 6G-FFT is responsible for the presence of the second chain. 6G-FFT uses 1-kestose as a fructose donor and puts the fructose via a β (2-6) linkage on the glucose residue of sucrose, forming neo-kestose. This trisaccharide is the first of the inulin neoseries, and can be elongated on both fructose residues with β (1-2) linked fructoses. Due to its structure, inulin in Lilicaeae family is not preferred by the industry [84].

Inulin is also synthesized by a number of bacteria, such as Streptococcus and Bacillus species, and fungi, such as Penicillium and Aspergillus species. In many of these, only one enzyme, fructosyltransferase (EC 2.4.1.9), is responsible for inulin synthesis. In *Aspergillus foetidus* a 1-SST was identified, indicating that fructan biosynthesis in this fungus is more similar to that of plants [84-86].

In contrast to plant inulin, bacterial inulin has a very high DP, ranging from 10,000 to over 100,000; moreover, this inulin is highly branched at $C_6$ [86] (≥15%). The fact that inulin has a small intrinsic viscosity in spite of its high molecular weight, and that it appears to adopt a compact, globular shape rather than a coil is another indication of its nonlinearity [81, 83].

Production of oligosaccharides by different reaction modes and enzyme forms obtained from microbial populations were studied by Japanese people mostly. *Pseudomonas* sp. and *Xanthamonas* sp. were mostly studied species.

In the study of Yun and coworkers (1997), fructooligosaccharides were produced by preparing fructosyltransferase from *Aureobasidium pullulans* KFCC 10254, extracellular endoinulinase from *Pseudomonas* sp. No. 65, and intracellular endoinulinase from recombinant *Escherichia coli*. Endoinulinase containing cells were also immobilized in alginate gel matrix. They found that mostly produced products have a degree of polymerization of 2 and 3, and no significant difference irrespective of enzyme sources and reaction modes [87].

In another two studies of the same researchers, batchwise and continuous productions were investigated. They reached 72 and 83% yield at the end of the soluble and immobilized form of the enzyme reaction at 55°C for 60 min, batchwise [88]. For continuous case the yield was 83% [89]. But in both cases, nearly 50% of the yield belonged to the products which had a degree of polymerization 2 and 3.

By using the enzyme of *Xanthamonas* species, batchwise production at 45°C reached 86% yield. But this time the major components were degree of polymerization 5 and 6 [90].

In the years 1999-2001, inulinase enzyme was found in other microbial species, *Saccharomyces cerevesiae* [91], *Aspergillus candidus* [92], and *Bacillus stearothermophilus* KP1289 [93]. But the enzyme obtained from these species used for fructose production.

Cho and coworkers produced FOS from pure inulin by a dual endoinulinase system originated from *Xanthamonas* sp. and *Pseudomonas* sp. They reached 92% yield using *Xanthamonas* enzyme to *Pseudomonas* enzyme in the ratio of 4:1 for 110 h [94]. In another study, they found that the distribution of the FOS components between pure inulin and chicory extract was not significantly different [95].

The term FOS is preferably used for the fructose oligomers up to maximum 7 units which contain one glucose unit linked α-1,2 to two 1-kestose ($GF_2$), three nystose ($GF_3$), four fructose units, $1^F$-fructofuranosylnystose ($GF_4$) etc., bound together by β(2-1) glycosidic linkages (Figure 6).

FOS exist naturally in many kinds of plants such as onions, asparagus roots, tubers of jerusalem artichoke and wheat, but also in banana, beer, burdock, chinese chives, garlic, endive, tomato, graminae (fodder grass), honey, oat, pine, rye, chicory, stone leak [16, 96, 97]. Chicory and JA contain a relatively high level of inulin and FOS. Industrially they have been produced from chicory.

The production processes of inulin and FOS are represented in Figure 7. Inulin production involves the extraction of the naturally occurring inulin from chicory roots, in a manner very similar to the extraction of sucrose from sugar beets (diffusion in hot water), followed by refining using technologies from the sugar and starch industries (e.g. ion-exchangers), and then evaporation and spray-drying [81].

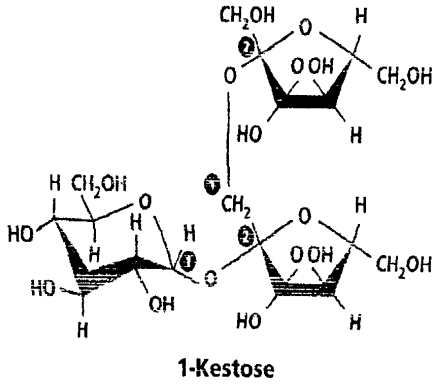


Figure 6. 1-kestose [98].

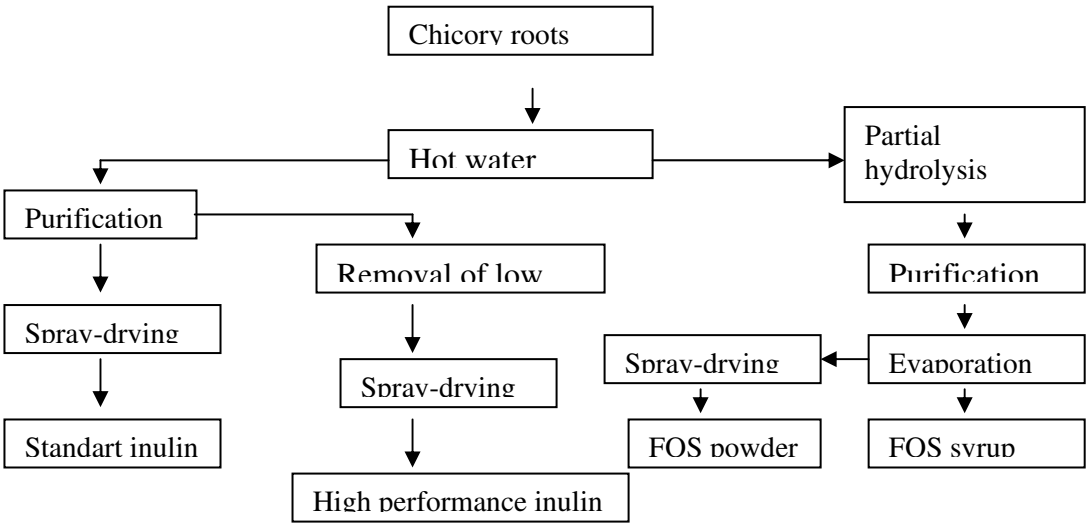


Figure 7. Industrial production process of chicory inulin and FOS [81].

In the production of FOS, inulin is hydrolyzed by enzymatic (using endo-inulinase, E.C.3.2.1.7) or acidic manner. When inulinase is used, FOS can be produced by one step to yield more than 90% [85, 99]. FOS mixture contains (α-D-Glu-(1-2)-[(β-D-Fru-(1-2)-]$^{n}$ where n= 2-8 and (β-D-Fru-(1-2)-[(β-D-Fru-(1-2)-]$^{n}$ where n = 1-8. The average DP of these oligosaccharides is between six and seven [71]. Acid hydrolysis of inulin displays several disadvantages such as undesirable coloring of the hydrolysate and formation of a difructose anhydrite [86].

Generally, the product with a degree of polymerization up to 60 is labeled as inulin (Raftiline), and up to 8 as FOS (Raftilose 95 and Nutra Flora). The inulin from which the small molecular weight oligomers have been eliminated is called inulin HP (Raftiline HP) [100]. Four companies in the world are known to produce inulin/FOS; two in Belgium, ORAFTI and Csucra, one in Holland, Imperial Suiker Unie, and a fourth in Korea, the Japanese sugar company, Meiji Seika Kaisha.

In addition to chicory inulin and its hydrolysate, the food industry also produces inulin and FOS synthetically. They are commercially synthesized from the disaccharide sucrose using sucrose 1$^{F}$-fructosyltransferases (EC. 2.4.1.99) from microorganisms such as *Aspergillus ficuum* and *Aspergillus niger*, *Aureobosidium sp*, *Arthrobacter sp*, *Fusarium sp.*, *Kluyveromyces marxianus* etc. [85, 86, 99], when glucose and small amount of fructose are formed as by-products [71, 74, 81]. This process yields a mixture of oligosaccharides containing one terminal glucose moiety linked to between two or four fructose units (α-D-Glu-(1-2)-[(β-D-Fru-(1-2)-]$^{n}$ where n = 2-4). The average number of moieties (DP) in these oligosaccharides is approximately four. When fructosyltransferase is used, no more than 55% sucrose can be used because of the inhibition of the enzyme activity by the resulting glucose molecules [101]. Therefore, to make syrup having a high content of FOS, some purification steps such as the use of ion-exchange resin are necessary to remove glucose and residual sucrose, resulting an increase in the cost.

Inulin is an attractive product with various applications in medicine (e.g. tests of renal clearance), in the pharmaceutical industry as inulin-drug conjugates, in

chemical industry for production of plastic polymers, glycerol, hydroxymethylfurfural, levunilic acid, mannitol, acetone-butanol mixture, 2, 3-butanediol, ethanol and in food industry as additive and dietary fibers especially for the production of fructose syrups obtained from chemical or enzymatic hydrolysis [83, 102, 103].

In the food industry inulin and FOS can be used for either their nutritional advantages or technological properties, but they are often applied to offer a dual benefit: an improved organoleptic quality and a better-balanced nutritional composition. Table 9 gives an overview of their applications in food and drinks [81]. Examples include drinks, yogurts, biscuits, infant formulas, table spreads, frozen desserts, meal replacers and other food products, food supplements, and feed additive in poultry [104, 72, 81]. In the dairy market, dietary products are showing the strongest growth, in particular diet yogurts with fruit.

Inulin and FOS give more crispness and expansion to extruded snacks and cereals, and they also increase shelf-life. They also keep breads and cakes moist and fresh for longer. Their solubility allows fiber incorporation in drinks, dairy products and table spreads. Inulin is also often used as a dietary fiber in tablets. Inulin and FOS allow the development of low-fat foods without compromising on taste or texture [82]. They impart a better-balanced round flavor and a creamier mouthfeel. In dairy mousses (chocolate, fruit, yogurt or fresh cheese-based), the incorporation of inulin improves the processability and upgrades the quality and stability. In frozen desserts, they provide an easy processing, a real fatty mouthfeel, excellent melting properties, as well as freeze-thaw stability. Inulin also has found application as a low calorie bulk ingredient and as a fiber in chocolate (e.g. without added sugar), often in combination with a polyol or with fructose. It also improves the stability of foams and emulsions such as aerated desserts, ice creams, table spreads and sauces. Inulin can therefore replace other stabilizers in different food products [81]. FOS depresses the freezing point in frozen desserts, and acts as a binder in nutritional or granola bars in much the same way as the sugar, but with the added benefits of fiber enrichment and other nutritional properties. In fact, it possesses technological

properties that are closely related to those of sugar and glucose syrup [105]. Its use is straightforward and requires only minor adaptation of the production process, if any. Therefore, it is an ideal ingredient for these properties.

Chicory inulin is available as a white, odorless powder with a high purity and a well-known chemical composition. Chicory FOS is as powder and colorless viscous syrup (75% dry substance). Their physico-chemical properties are summarized in Table 10.

Table 9. Overview of food applications with inulin and FOS [81].

| **Application** | **Functionality** | **Dosage level of FOS (%w/w)** | **Dosage level inulin (% w/w)** |
|---|---|---|---|
| Dairy products | Sugar and fat replacement<br>Synergy with sweeteners<br>Body and mouthfeel<br>Foam stability<br>Fiber and prebiotic | 2-10 | 2-10 |
| Frozen desserts | Sugar and fat replacement<br>Texture and melting<br>Synergy with sweeteners<br>Fiber and prebiotic | 5-12 | 2-10 |
| Table spreads | Fat replacement<br>Texture and spreadability<br>Emulsion stability<br>Fiber and prebiotic | | 2-10 |
| Baked goods and breads | Fiber and prebiotic<br>Moisture retention<br>Sugar replacement | 2-25 | 2-15 |
| Breakfast cereals | Fiber and prebiotic<br>Crispness and expansion | 2-15 | 2-25 |
| Fillings | Sugar and fat replacement<br>Texture improvement | | 2-30 |
| Fruit preparations | Sugar replacement<br>Synergy with sweeteners<br>Body and mouthfeel<br>Fiber and prebiotic | 5-50 | 2-10 |
| Salad-dressings | Fat replacement<br>Body and mouthfeel | | 2-10 |
| Meat products | Fat replacement<br>Texture and stability<br>Fiber | | 2-10 |

Inulin has a bland neutral taste, without any flavor or aftertaste. Standard inulin is slightly sweet, whereas high performance inulin is not. It is moderately soluble in water and brings a rather low viscosity. As far as fat replacement is concerned, high performance inulin shows about twice the functionality of standard chicory inulin. Inulin works in synergy with most gelling agents, e.g. gelatin and alginate [105].

Fructo-oligosaccharide is much more soluble than inulin and sucrose. In the pure form it has a sweetness of about 35% in comparison with sucrose or glucose and, unlike such sugars, can be used also by diabetic people [81, 82]. Its sweetening profile closely approaches that of sugar, the taste is very clean without any lingering effect and it also enhances fruit flavors; sweetness decreases with increasing DP [22]. Fructo-oligosaccharide shows good stability (highly stable in the normal food range of pHs (4.0-7.0)) during the usual food processes (e.g. during heating) [81].

Table 10. Physico-chemical characteristics of chicory inulin and FOS [81].

| | **FOS** | **Standard Inulin** | **Inulin HP** |
|---|---|---|---|
| Chemical structure | $GF_n + F_n$ (2<=n<=7) | $GF_n$ (2<=n<=60) | $GF_n$ (10<= n <= 60) |
| Average dp | 4 | 12 | 25 |
| DM (%) | 95 | 95 | 95 |
| Inulin content (% on dry matter) | 95 | 92 | 99.5 |
| Sugars content (% on dry matter) | 5 | 8 | 0.5 |
| pH (10% w/w) | 5-7 | 5-7 | 5-7 |
| Sulphated ash (% on dry matter) | < 0.2 | < 0.2 | < 0.2 |
| Heavy metals (ppm on dry matter) | < 0.2 | < 0.2 | < 0.2 |
| Appearance | White powder | White powder | White powder |
| Taste | Moderately sweet | Neutral | Neutral |
| Sweetness (v. sucrose=100%) | 35 % | 10 % | None |
| Solubility in water at $25^0$C (g/l) | > 750 | 120 | 25 |
| Viscosity in water (5%) at $10^0$C (mPa.s) | < 1.0 | 1-6 | 2.4 |
| Functionality in foods | Sugar replacers | Fat replacers | Fat replacers |
| Synergism | Synergy with intense sweeteners | Synergy with gelling agents | Synergy with gelling agents |

The DP, as well as the presence of branches, is important properties since they influence the usage of inulin and FOS in the food industry and functionality to a striking extent; for example sweetness increases with decreasing DP. According to technological and functional aspect, the DP should be in between 3-6. The DP varies according to the plant species, plant's life cycle, weather and storage conditions, and the physiological age of the plant, harvest date, extraction and post extraction processes [105].

There was a general agreement among the AOAC International Workshop, 1995, participants that inulin and FOS be included in the prebiotic soluble dietary fiber complex [77]. Studies designed to determine the prebiotic effect of inulin and FOS are summarized in Table 11. Average daily consumption of inulin has been estimated to be between 1-4 g in the US [106], 3-11g in Europe [106] and 2- 8g in the North America [16, 97]. Doses of 4-5g/d are efficient in stimulating the growth of bifidobacteria classified as potentially beneficial for health [6, 15]. A reasonable caloric value for inulin and FOS is estimated to be 1.5 (6.3) kcal/g (kj/g) or ˜38% that of a digested hexose molecule [100]. Both inulin and FOS have GRAS status by the authorities in Australia, Canada, USA and Japan.

Table 11. Summary of studies designed to determine the prebiotic effect of inulin and FOS (72).

| Oligosaccharide | Mode of study | Evidence of prebiotic effect | Reference |
|---|---|---|---|
| FOS, GOS, TOS | In vivo gnotobiotic rats | High increases in bifidobacteria numbers with FOS and TOS | [107] |
| FOS (from sucrose) | In vivo rats | Increase in lactic acid bacteria after 2 weeks, but in the long-term any effect was lost | [108] |
| Inulin | In vivo DSS induced colitis rats | Decrease in luminal pH between left and right colon | [109] |
| Inulin | In vivo DSS induced colitis rats | Increase in lactobacilli | [110] |
| Inulin | In vivo eight healthy humans | Significant increase in bifidobacteria established by FISH | [111] |
| FOS | In vivo double-blind placebo controlled IBS patients | No therapeutic effect at 6 g/d. | [112] |
| FOS (from sucrose) | In vivo forty healthy humans | Significant increase in bifidobacteria levels without excessive gas production at 10g/d | [113] |
| Inulin and FOS | In vivo eight healthy humans | 15g/d inulin to oligofructose led to bifidobacteria becoming predominant in faeces | [114] |
| Inulin and lactose | In vivo twenty-five elderly constipated humans | Significant increase in bifidobacteria, decreases in enterococci and fusobacteria. Better laxative effect than lactose | [115] |
| FOS | In vivo double-blind placebo controlled crossover study of thirty healthy humans | Significant increase in bifidobacteria established via FISH at 7g/d, no change in total bacterial levels | [116] |
| FOS (from sucrose) | In vivo ten healthy adult humans | Significant increase in bifidobacteria, some increase in lactobacilli | [117] |
| BFM and BFM plus inulin | In vivo twelve healthy humans | Increase in bifidobacteria with BFM but addition of inulin did not enhance effect | [118] |
| Inulin | In vivo six healthy humans (low stool frequency) double blind placebo controlled crossover study | Significant increase in stool frequency and faecal bulk | [119] |
| Inulin and FOS | In vitro human faecal flora batch cultures | Significant increase in bifidobacteria, suppression of E. Coli and clostridia | [120] |
| FOS (from sucrose) | In vivo twelve healthy adult humans | Significant increase in bifidobacteria, no change in total bacteria levels | [121] |

Table 11 (continued)

| Oligosaccharide | Mode of study | Evidence of prebiotic effect | Reference |
|---|---|---|---|
| FOS and fourteen other carbohydrates | In vitro batch cultures of seven Bifidobacterium isolates | Best supported growth of test bacteria on oligofructose and GOS | [122] |
| Inulin, rye, wheat and oat brans | In vitro human faecal batch cultures | Highest decrease in pH with inulin and highest increase in butyrate, very fast fermentation and high gas production | [123] |
| FOS | In vitro human faecal flora continuous culture and in vitro 3-stage gut model | Significant bifidogenic effect compared to sucrose and inulin | [57] |
| FOS | Sixteen strains lactobacilli eight bifidobacteria | Twelve of sixteen lactobacilli and seven of eight bifidobacteria strains fermented FOS | [124] |
| FOS | In vitro human faecal flora continuous culture | Increases in bifidobacteria. Lactobacilli out competed bifidobacteria at pH 5.2-5.4 | [125] |

Unless otherwise stated, observations on the bacterial flora listed in Table 11 were obtained via microbial culture techniques.

To be a prebiotic, it must be demonstrated that these compounds were metabolized by probiotic microorganisms as stated before. There are several ways used in the literature to demonstrate the fermentation of fructo-oligosaccharides. Commercial FOS can be purified by using chromatographic methods as much as possible (generally purified FOS means; FOS containing 2.3% glucose and 2.3% fructose) [126], or if it is synthesized enzymatically from sucrose it can be radiolabelled. Investigation of an increase in growth rate in FOS-containing medium versus control or formation of colored colonies demonstrates the fermentation. Strains that fermented FOS grow as colonies as surrounded by a yellow zone (>3 mm) against a purple background [127]. Non-fermenting colonies produce smaller white colonies without a yellow zone. In that way, the only information obtained is whether the strain ferments that compound or not, the fermentation can not be quantified. In the method of investigating the growth rate, optical density of the medium is measured. The control for all those experiments can be either a MRS basal media + same amount of glucose and fructose in the purified FOS or MRS media itself if FOS was added directly onto this media in the experiments [126, 124, 127]. Generally, if the FOS-containing food is to be analyzed, second way has been chosen in the studies.

In fact, relatively little is known about which strains actually metabolize these materials. Because commercial oligosaccharide preparations often contain glucose, fructose, sucrose, or other fermentable sugars, it has been difficult to establish that growth in FOS-containing medium is due to actual utilization of FOS [126, 128, 129]. It was found that, *L. plantarum (37, 73, 80, MR 240, DSM 20174, DSM 20246, NCIMB 1193, and HU)* [124, 127], *L.casei (MR191* and *685)* [124], *and L. paracasei subsp. paracasei, L. brevis,* and *Pediococcus pentosaceus* [16] fermented FOS, whereas *Lactobacillus strain GG*, one of the best studied probiotic strains, 8 strains of *E.coli,* and *Salmonella spp.* were found to be non-fermenter [124]. Results suggest that FOS utilization did not require an induction period and that FOS was as good a substrate as glucose in supporting growth [124].

As more and more scientific data become available, the nutritional benefits of inulin and FOS become further apparent. There was a general consensus that there is a strong evidence for a prebiotic effect of inulin-type fructans in human subjects and for the impact that they have on bowel habit; there is promising evidence that consumption of inulin-type fructans may result in increased Ca absorption in man; there are preliminary indications that inulin-type fructans interact with the functioning of lipid metabolism and a preventing effect against colon cancer (Table 12).

Table 12. Consensus on the different functional food effects of inulin-type fructans in decreasing order of established evidence in human studies [130].

| **Effect** | **Evidence (in human subjects)** |
|---|---|
| Prebiotic and interaction with intestinal flora | Strong |
| Regulation of bowel habit, stool bulking, and increase of stool frequency | Strong |
| Increased mineral absorption | Promising |
| Impact on lipid metabolism | Preliminary, data still inconsistent |
| Colon cancer | No human data available (experimental animals, preliminary) |

## 1.3 Jerusalem Artichoke

Jerusalem artichoke (*Helianthus tuberosus L.*) is a hairy, tuber-bearing perennial of the same *Compositae* family as sunflower (*Helianthus annuus L.*). It is a native of the North America regions around the 36th parallel and has been grown in Europe since the seventeenth century. Starting from the beginning of twentieth century it was cultivated on a large scale in France, Spain, and Germany. Although it can be cultivated especially in Middle East, jerusalem artichoke is not consumed so much in our country.

The jerusalem artichoke is a versatile, low-requirement plant suitable also for marginal lands, potentially achieving high yield of biomass in the form of tubers and stems and shows good frost and drought tolerances, as well as resistance to pest and diseases. Such a resistance and adaptability of jerusalem artichoke can be explained by the fructan metabolism since these plants are able to accumulate fructans instead of starch as reserve carbohydrates. Fructan metabolism is the ability of the species to modify the degree of polymerization of the fructan pool [131]. Jerusalem artichoke does not contain bitter taste compounds and extraction steps can be omitted when palatable functional ingredients are produced [132]. And because of the absence of interfering components they can be easily extracted and processed to purified products.

Jerusalem artichoke is propagated by tubers, which should be planted as early as possible in the spring. Tubers begin to form in August, and they could be harvested in winter and even early spring, so that a very long processing period would be possible [132]. The maximum accumulation of these carbohydrates occurs up to the milky phase. In the summer and dry climatic conditions the reduced demand of sucrose for vegetative growth leads to a rapid increase in the amounts of soluble carbohydrates [133]. During this period until the end of September, 70-80% of the photosynthesis product exceeding growth requirements is stored in the stems and the

remaining 20-30% in the tubers. After flowering, during October, the reserves accumulated in the stalks are transferred to the tubers. During that translocation time (30-40 days) the total amount of WSC per plant is constant, and only afterwards, in November, for some cultivars, if the temperature remains mild another small increase of insoluble carbohydrates in the tubers may occur, due to the direct transfer of photosynthate from the aging leaves to the same tubers. When the rainfall was sufficient to promote increased photosynthesis, further increase of sugar was determined in the tubers. In an early-maturing cultivar, the sugar accumulation and distribution were found similar [134].

Fructan synthesis and accumulation in jerusalem artichoke is mostly confined to the underground tubers. In the subsequent dormant period hydrolysis and depolymerization of fructans takes place. These events are catalyzed by the combined action of FH and 1-FFT, and results in a marked increase in concentration of small polymers of DP 3-6 [135]. During polymer hydrolysis fructose is produced, however, practically no free fructose is found in dormant tubers. Furthermore, during dormancy, the total hexose content remains fairly constant. The fact here is explained by the synthesis of sucrose from fructan hydrolytic products and subsequent synthesis of low DP fructans from sucrose. Thus, tubers appear to have the capacity to convert free hexoses to sucrose at all times from their initiation to sprouding. The activities of the enzymes of fructan metabolism also vary during dormancy; FH activity was found to be increasing at the end of dormancy and that of FFT was decreasing during it [136]. It was shown that the plant enzymes catalyzing the synthesis of fructan in chicory or jerusalem artichoke are located in the vacuole and use sucrose as the primary substrate. In stored jerusalem artichoke, SS is the enzyme for sucrose synthesis. For SS to be involved in the synthesis of sucrose during dormancy, fructose produced by fructan hydrolysis, must move from the vacuole into the cytosol. The newly synthesized sucrose in turn moves into the vacuole where it serves as a preferred acceptor for 1-FFT [135]. Thus the net result is an increase in the level of low DP members (DP 3-6) of the fructan series, as it was described by Jefford and Edelman (1968) in their studies of fructan mobilization during dormancy

[137]. Breakdown of fructan often precedes growth, for example in the root of chicory and jerusalem artichoke during dormancy [136].

Jerusalem artichoke is known to show a large interannual and geographical variation in productivity. Performance of JA depends on several factors, such as photosynthetically active radiation, evapotranspiration, nutrition, etc. [138]. D'egidio et. al., reported the influence of enviromental conditions on WSC and fructans content and the high temperatures of southern Italy caused a remarkable reduction in fructans accumulation [132]. It should be noted that, for the cereal species considered, the total fructose content of the hydrolyzed WSC is about 70% only in jerusalem artichoke and chicory roots [132]. Although, the dry weights were found as unchanged during winter (around 20% fresh matter) [139], it is well known that there is a gradual decrease in the average degree of polymerization of the fructosides in the tuber during winter storage. Furthermore, most of the polymerized fructose was lost during sprouding of the tubers, much of it providing substances for the early growth of the daughter plants [135].

The DP of inulin varies depending on the prevailing agricultural conditions during growth (climatic and soil parameters), the cultivar and on the harvest date. In this crop, low nitrogen fertilization increases the fructose content of the stems. As has been cited in the literature by Chabbert et.al., the fructose/glucose (F/G) calculation gave decreasing values with a maximum of 11 from the beginning of the harvest in September to a minimum of 3 as early as December [140]. It is important to note that the water solubility of oligo- and polyfructans decreases with an increasing degree of polymerization. Therefore, maturity and storage conditions of the JA tubers will influence the extractability of the fructans. The caloric value is also extremely dependent upon storage time, and may range from 42 to 420 kJ per kg. This compares favorably to the potato which is approximately 420 kJ per kg [138].

The composition of the jerusalem artichoke tubers is shown in Table 13. The carbohydrate portion of the JA tuber constitutes approximately 75% of the dry matter (15-20% of wet weight), and is composed of polyfructans (termed inulides). Native

chicory inulin (i.e. extracted from fresh roots, taking precautions to inhibit the plant's own inulinase activity as well as acid hydrolysis) has an average DP of 10-20 [78]. The JA tuber is a good source of B vitamins, pantothenic acid, potassium, and phosphorus. Tubers also contain large quantities of vitamin A, iron and calcium. The limiting essential amino acid for the JA is methionine (58% of that in egg); while most other amino acids are present in excess of 100% (exceptions are found for phenylalanine, tyrosine, isoleucine, and leucine which range from 80-95%). In comparison protein score for JA is found to be greater than, or equivalent to most other traditional food crops (i.e. corn, wheat flour, and beans) [141].

JA tubers (Figure 8) contain native inulinase which may be used to break down poly- and oligo fructans. Two different conditions for optimal tuber inulinase activity have been reported. It was stated that 55-56°C and a pH of 6-6.5 (pH of the tubers) results in the greatest extent of polyfructan hydrolysis [142]; however, another book indicates that these values are 40°C and pH 5.1, respectively [143]. Activity at 56°C is advantageous since this temperature will partially prevent contamination of the pulp; however, 40°C will not. The time for complete hydrolysis of the inulides is dependent upon the DP of polyfructans in the tuber, and this value is subject to a high degree of variation [141].

Table 13. Composition of jerusalem artichoke tubers (*Content in solid matter) [144].

| | |
|---|---|
| Water | 75-81 % |
| Proteins | 10-15* % |
| Saccharides | 75-80* % |
| Lipids | 1* % |
| Ash | 5* % |
| Fiber | 4-6* % |
| Calcium | 23 mg/100 g |
| Phosphorus | 99 mg/100 g |
| Iron | 3.4 mg/100 g |
| Zinc, Ascorbate, Riboflavin, Niacin | Traces |

Figure 8. Jerusalem artichoke tubers

JA tubers have been reported to exhibit discoloration reactions during processing. The catalytic action of PPO is connected to undesirable browning and off-flavor generation in stored and processed foods of plant origin. In this respect, the extensive discoloration encountered during the processing of jerusalem artichoke tubers for the production of inulin hydrolysate has been linked to the presence of a highly active PPO system. In the research of Ziyan and Pekyardımcı [145], several inhibitors were used to stop PPO activity including L-cysteine, L-ascorbic acid, sodium azide, sodium diethyl dithiocarbomate, thiourea and citric acid. The most effective inhibitors of PPO were found to be sodium azide and thiourea for both flesh and skin PPO. Although the amount of inhibitor was not stated, they found 50% and 89% activity remaining after addition of citric acid for skin and flesh PPO, respectively.

High biomass yields per hectare, coupled with a favorable composition and substantial level of carbohydrates, give the JA a number of important applications. The simplest and original use of this crop was a foodstuff for humans and livestock. Either the fleshy tubers or the fibrous tops of the JA may be used as animal feed whereas human consumption is primarily limited to the tubers. However, many other industrial uses have been suggested and studied. The greatest extent of these applications have been reported to be in the production of high-fructose or pure fructose syrups or ethanol [134]. The hydrolysis of inulin sugar from JA produces syrups with D-fructose content over 75% [103]. Chemical hydrolysis of inulin to fructose displays several drawbacks, and much attention was paid to the use of

inulinase (E.C. 3.2.1.7) for enzyme hydrolysis [146]. Early harvested tubers, before inulin depolymerization and containing high-molecular-weight inulin, are better for the production of high-fructose-containing syrup after hydrolysis, such as HFCS or UHFCS [147]. Late-harvested tubers containing low-molecular-weight inulin are well suited for fermentations, or for isolation of FOS [139].

In addition to these major uses, the JA has also been studied as a substrate for the production of acetone and butanol, mixture of acetone-butanol-ethanol, fodder yeast, beer, lactic acid, propionic acid, mannitol, and pectic substances [141].

The JA is an excellent crop for inulin production and the US, Russia and some European countries use it in their food and pharmaceutical industries as a raw material because of its valuable properties defined. In terms of food processing, the tubers of the JA have been utilized in the manufacture of bread sticks, cookies, macaroni and noodles. Cooking the tubers is primarily performed so as to hydrolyze the long-chain carbohydrates. However, a number of disadvantages are inherent to the use of tubers in cooking. During the process of cooking, it is important to note that cell rupture is extensive and non-soluble matter (such as cellulosic material) is suspended in the liquid phase. Problems in the pumping of this mash to bioreactors and subsequently to the distillation columns on a commercial scale have been reported. It has been found that this non-soluble material (mostly the skin of tubers) makes up 2.5-4% of total tuber mass depending on the temperature used for cooking. Though often referred to a potato substitute, the organoleptic properties of JA tubers are considered to be much different. In addition, inulides do not swell like starch, and cooked tubers remain extremely watery. A crisp brown coat does not developed upon frying as it does for the potato, and required cooking times are much shorter (about 10 min) after which the tubers become transparent and soggy [141].

## 1.4 Sugar Production and Extraction of Fructo-oligosaccharides

Sugars have been produced from sugar beet by normal diffusion in the current industry. Grated sugar beets (4-8 mm width, 10 cm length) have been extracted with water at 70°C during 1 hour in a counter-current manner. Syrups have nearly 14% sugar before whitening, evaporation and drying. In the extraction, 120kg water is used for 100kg sugar beet. Production amount and cost of sugar beet are 13,517,241 tons and 108 376 TL/kg, whereas the corresponding values are 60 tons and 603 217 TL/kg for jerusalem artichoke, respectively [148].

In the production of sweetening syrups, the extraction of jerusalem artichoke is an important process step that greatly influences sugar recovery. Upon extraction with as little solvent as possible, extensive desugarization of the jerusalem artichoke should be achieved. In literature, information for the extraction of jerusalem artichoke is scarcely available.

The first study on the extraction of jerusalem artichoke was done by Conti in 1953 [149]. In the study, methods of analysis for the tubers of jerusalem artichoke were discussed, the variations of dry matter, carbohydrate, and ash content of the tubers were analyzed. The JA tubers harvested in February, April, and December of the same year (1952) was used in analysis. The highest total dry matter content was found as 18–26% in February-harvested tubers, while the least value (16–20%) was found by April-harvested tubers. The ash content was found relatively independent from the dry matter. The study also provides a syrup production by extracting the carbohydrates via normal diffusion using the same equipment as for sugar manufacture. The solvent had a temperature of 80°C, and the contact time was an hour. The syrup yield was 80% yield and a pH was 4-4.4. Economic considerations were also investigated for the production of the syrup, and the process found costly.

In another study [147], an atmosphere of sulfur dioxide was utilized in order to lower the pH of the water to 1-2, and also to prevent contamination. Although lower temperatures (up to 70°C) and lower contact time (30 min) were used, the obtained extraction yield was higher (95%). In that research, it was reported that the rate of extraction increases with increasing temperature up to about 60°C, but no differences were observed with higher temperatures. The syrup content after acid hydrolysis was measured as glucose and fructose, and 67% of the total carbohydrate content was found as fructose and the remaining as glucose.

Schorr-Galindo et.al. (1997) performed the extraction with boiling water by submitting the crushed tubers in the study of crop growth, development and yield of jerusalem artichoke [139]. They found that the degree of polymerization of inulin varied, depending on the cultivar and on the harvest date. The F/G calculation gave decreasing values, with a maximum of 11 from the beginning of harvest in September to a minimum of 3 in February. Thus, they concluded that September-harvested tubers contained a greater amount of highly polymerized sugar fractions, and they should be used after hydrolysis, but spring harvest always lead to low-molecular weight inulin extracts which were well-suited for fructo-oligosaccharide isolation. They also found that storage time in soil strongly affected on the sugar content of these crops.

In a more recent study [132], extensive investigations had been carried out by the authors both on jerusalem artichoke as well as barley, drum and bread wheat. The accumulation of fructans in the stems and tubers were obtained by a water extraction at 105°C for 2 h, after a preliminary extraction by ethanol at 96°C (two-step extraction). The achieved yield was 90% for JA. It was found that, the high lighting condition increased the photosynthetic activity while poor summer rains reduced the plant growth without stopping photosynthesis for all the crops investigated. These conditions, particularly useful for European countries, favored the accumulation of fructans.

For continuous preparation of fructose syrups from jerusalem artichoke tubers, Wenling et al. (1999) used 2 l of water for 500 g of tuber as dry powder. The extraction was carried out at 100°C during 40 min. Using a 4.5% (w/v) fructan solution from jerusalem artichoke tuber as substrate, in a continuous bed column reactor packed with the immobilized inulinase beads, the maximum volumetric productivity was obtained with fructan hydrolysis of 75% [103].

## 1.5 Ultrasonication and Its Effects on Extraction

Ultrasound is defined as sound waves with frequencies above that of human hearing [150]. These waves can be propagated in a liquid media as alternating compression. If ultrasound has sufficient energy, a phenomenon known as cavitation occurs. Cavitation involves the formation, growth, and rapid collapse of microscopic bubbles. Based on theoretical considerations, extremely high temperatures and pressures are momentarily delivered to the liquid media during the collapse of bubbles [151]. Electrical discharge and production of free radicals have also been associated with extreme conditions occurring inside the collapsing bubble [152].

Ultrasonic techniques are finding interesting use in the food industry for both the analysis and modification of foods. Low-intensity ultrasound is a non-destructive technique that provides information about physicochemical properties, such as composition, structure, physical state and flow rate [153]. Low intensity ultrasonic waves can modify cellular metabolism via enhancing the activity of an enzyme as it has been shown in the study of Stephen et.al. about invertase activity towards sucrose [154].

High-intensity ultrasound is used to alter, either physically or chemically, the properties of the foods. Ultrasonication-assisted extraction is the application of high-

intensity, high-frequency sound waves and their interaction with materials [155, 156]. Both low and high intensity ultrasound waves can also result a significant enhancement of reaction rate due to improvement of the mass transfer of reagents and products through the boundary layer or through the cellular wall or membrane [157] or probably due to a reduction in substrate inhibition and aggregation based on hydrogen bonding of molecules [158]. In the case of raw plant tissues, ultrasound has been suggested to disrupt plant cell walls thereby facilitating the release of extractable compounds and enhance mass transport of solvent from the continuous phase into plant cells [159, 160, 161]. In the study of Carcel et.al. [160], application of the ultrasonic waves with intensity of 11.5 $W/cm^2$ during 45 min resulted in an increase of 117% for water diffusivity and 137% for dry matter diffusivity compared with static diffusion. In another study, application of 5 $W/cm^2$ ultrasonic waves for half an hour improved the yield of oil and lowered the percentage of heavy compounds extracted [161]. In addition, several studies have demonstrated that high intensity ultrasonic waves resulted an inactivation or denaturation of the enzyme such as glucose-6-phosphate dehydrogenase [162], chymotrypsin [158], and invertase [154] by cavitation. In those studies, it was reported that the effect of ultrasound dependent on the molecular weight, structure, initial enzyme concentration, substrate concentration and properties of the reaction media (e.g., pH). Thus calling the frequency of the ultrasound as low or high intensity is also dependent on those parameters. The conditions required for enzyme inactivation in those studies were 1 $W/cm^2$ during 20 min at 50°C [162]; 0.9M sucrose concentration, 62 $W/cm^2$ ultrasound applications during 30 min at 40°C for chymotrypsin [158]; and pH of 5.4, 21 kHz, 55 $W/cm^2$ during 4 hours for invertase [154]. Due to the capability of inactivation of certain enzymes, especially thermotoleranced ones, and microorganisms, mainly bacterial spores, the main area of application of the ultrasound waves in the food industry is food preservation [150, 163, 164]. Usage of ultrasonication instead of heat treatments in food preservation have several advantages including no requirement of extremely high temperature usage, no adverse effect on flavor, taste, and nutritive value of foods especially caused from heat treatments, and also capability of inhibition of spore-forming microorganism growth that has high resistance to heat treatments.

In the extraction processes, it may be an alternative to the traditional method, in addition to those advantages stated above, it reduces the required time, increases the extraction efficiency, and may reduce the enzyme activity that produces undesirable components. But in literature there are only two studies present on the effect of ultrasonication on production of inulin and fructo-oligosaccharides. One of that examined the effect of ultrasonication on the release of fructosyltransferase from *Aureobasidium pullulans* CFR 77, and subsequently on the enzymatic production of fructo-oligosaccharides from sucrose. The amount of fructo-oligosaccharides produced ranged from 57 to 59% by ultrasonication of the cells at acoustic power of 20W for 9 min [165].

In the other study [166], the researchers optimized the conventional inulin extraction, and then compared the results of those with the ones obtained by direct (using probe horn) or indirect sonication extraction (with ultrasonic cleaning bath). To optimize the conventional extraction of inulin, various combinations of pH (7-9), time (2-40 min), temperature (30-50°C), and solvent to solid ratio (4-6) were used. The jerusalem artichoke powder was obtained by peeling, slicing, drying, milling until the whole sample passed through a 0.125 mm sieve. They used central composite design and response surface methodology for experimental design. Based on analysis, the optimal conditions for maximizing inulin extraction yield (83.6%) were at neutral pH for 20 min at 76.65°C and solvent to solid ratios of 10.56:1 (v/w). The researchers used ultrasonic equipment (150W), producing ultrasound with 59 KHz frequency for indirect sonication, and 20 KHz for direct sonication. They obtain 84% yield via indirect sonication, and 85% yield via direct sonication. They found that the extraction rate of the direct ultrasound-assisted process was about two times faster than that of conventional method. They approached maximum yield at about 8 min for direct sonication, and 10 min for indirect sonication. They did not quantify the product profile of the extracts since they analyzed the extracts via thin layer chromatography. By using the area of the peaks of glucose, fructose, sucrose, 1-kestose, and nystose, they concluded that direct sonication increased the oligosaccharide yield, since it degraded inulin partly.

## 1.6 Microwaving and Its Effects on Extraction

Microwaves activate the water molecules or particles of food, causing heat by friction which cooks or reheats the food. It minimizes food shrinkage and drying out, since food is cooked in its own juices, its flavor and goodness are retained. Also, the method is economical on electricity and labor, and it is recommended when compared with other traditional cooking methods. But the method may not be useful for all foods. In the literature, microwaving has been used for several reasons, including increasing the nutritional value of the food, inactivation of the enzyme that produces undesirable compounds in the food [167–172].

In one of the studies [167], the nutritional composition of chickpea as affected by microwaving and other traditional cooking methods was investigated. The authors analyzed the effect of cooking treatments on fat, total ash, carbohydrate fractions (reducing sugars, sucrose, raffinose, and verbascose), antinutritional factors (trypsin inhibitor, haemagglutanin activity, tannins, saponins and phytic acid), minerals and B vitamins. Boiling was achieved via cooking in tap water at 100°C on a hot plate for 90 min. Autoclaving was applied at 121°C for 35 min. Microwaving was applied for 15 min. Based on the results, microwave cooking was recommended for chickpea preparation, not only for improving nutritional quality (by reducing the level of antinutritional and flatulence factors as well as increasing in-vitro protein digestibility and retention rates of both B-vitamins and minerals), but also for reducing time.

In another study [168], the effect of different processes used in modern large-scale service systems and the food industry on retention of folates in vegetables was investigated. The concentration of folates present in raw samples of peas, broccoli and potatoes was measured during different cooking methods, warm and cold holding and reheating. The following decreasing order in folates retention, on dry matter basis, was obtained compared to raw potatoes during heat processing: sous-

vide: (103%), boiling (72-59% (unpeeled and peeled)) and oven-baking (63%) and compared to raw green peas during heat processing: boiling (77%), microwaving (75%), and steam boiling (73%) and blanching (71%). Thus it was concluded that microwave cooking was another suitable method for cooking when compared with the others.

One of the examples of denaturing the enzyme via cooking is the study of Song (2006) [169]. In that study, the effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables were investigated. Glucosinolates are chemically stable until they come in contact with the enzyme myrosinase, which converts them to isothiocyanates. Cooking at high temperatures denaturates myrosinase in vegetable material, resulting in lower conversion of glucosinolates to isothiocyanates when chewed. They found that microwave cooking (5 min, 1000W) produced significant decreases of isothiocyanates content.

In the last 15 years there has been an increased interest in using microwave-assisted extraction techniques. Important parameters are the nature of the solvent and volume, temperature, time and particle size of the matrix. Through numerous examples, it is demonstrated that microwave-assisted extraction allow reduced solvent consumption and shorter extraction times, while the extraction yield is equivalent to or higher than those obtained with conventional methods [173]. The first papers to report the use of microwave-assisted extraction for natural products were published by Ganzler and co-workers [174, 175, 176] and concerned the extraction of vicine and convicine from faba beans: these toxic pyrimidine glycosides preclude the use of faba beans as a source of nutritional proteins. Ground beans were suspended in a methanol:water mixture (1:1, v/v), and the suspension was subjected to two successive microwave irradiations (30s each) with a cooling step in between. No degradation was observed under these conditions, but further irradiation was found to decrease the yield. The yield obtained was 20% higher than with the conventional Soxhlet extraction method.

In another study, five terpenic compounds associated with grape aroma were extracted from must samples by microwave-assisted extraction [177]. Four variables

(solvent volume, temperature, time and amount of sample) were optimized by means of two- and three-level factorial designs. The applied power was fixed as 475 W. The final optimized conditions were as follows: 5 ml sample amounts extracted with 10ml of dichloromethane at 90°C for 10 min with the microwave power set at 50%.

Recently, a system has been developing simultaneously to saponify and extract ergosterol by microwave-assisted extraction [178]. The determination of this compound in filtered air or dust can be used as an indicator of fungal contamination. The samples were placed in culture tubes containing 2ml methanol and 0.5ml 2M sodium hydroxide. Microwave irradiation was applied at 375W for 35s and the samples were cooled for 15 min before neutralization. It was demonstrated that the yield was similar to or even higher than that obtained with the traditional methanolic extraction followed by alkaline saponification.

The application of microwave energy to the extraction of taxanes from taxus biomass was reported by Incorvia Mattina et.al. [179]. Various parameters, including temperature, extraction time, solvent choice, and water content were investigated. Recoveries of taxane reached 100% of the conventional method when the biomass was freeze-dried to less than 10% moisture and pre-soaked with water prior to extraction using 95% ethanol. The temperature was set at 85°C and microwave-assisted extraction was performed for 54s. The extracts were quantitatively and qualitatively equivalent to those obtained with the conventional extraction method, but with considerable reduction of both extraction time and solvent consumption.

The extraction of glycyrrhizic acid from licarice food was studied by Xuejun et.al. [180]. Various experimental conditions, such as extraction time, ethanol and ammonia concentration, liquid/solid ratios, pre-leaching time and material size were investigated to optimize the efficiency of the extraction. The assist of microwaves 700W, 15s application) to the conventional extraction methods including extraction at room temperature, the traditional Soxhlet extraction, heat flux extraction and ultrasonic extraction were determined. The microwave-assisted extractions provided equal extraction efficiencies, reduced solvent.

No study was found in the literature investigating the effect of microwaving on extraction of jerusalem artichoke tubers.

## 1.7 Objectives of the study

The objective of this research was to study the effects of process variables, such as solvent to solids ratio, temperature, processing time, harvest date and storage time on the extraction of sweetening syrups from jerusalem artichoke with as high as possible fraction of monosaccharide units in the degree of polymerization range of 3-6. Fort his purpose, conventional water-bath extraction was studied batch-wise. The effect of citric acid addition to improve color and acid hydrolysis, and degree of assistance to extraction that could be obtained by microwaving and ultrasonication was also studied. Additionally, prebiotic properties of the syrups produced were evaluated by growth rate of a probiotic microorganism chosen as *Lactobacillus plantarum* NCIMB 1193.

# CHAPTER 2

# EXPERIMENTAL

## 2.1 Materials

Materials used in this study were jerusalem artichoke tubers, some chemicals, and a probiotic bacterium.

Since the composition of jerusalem artichoke tubers changes with storage time, they brought from Beypazarı (Ankara) to the laboratory in the same day as they harvested, and then the fresh-case experiments were done. For experiments in which stored jerusalem artichokes were used, they were wrapped with paper towel to delay spoiling, and stored in a plastic bag in a refrigerator at 4°C during the storage time to be studied.

Chemicals were citric acid (C1857), potassium sodium tartrate (S6170), sodium carbonate (S7795), sodium bicarbonate (S6297), sodium sulfate (S5647), copper-II-sulfate (C1297), ammonium molibdate (A1343), sodium arsenate dibasic heptahydrate (A6756), glucose (G7528), fructose (F0127), sucrose (S7903), 1-kestose (F72555), nystose (F56218), MRS broth (M110661), yeast extract (70161), sodium acetate trihydrate (S7670) obtained from Sigma, Fluka, and Merck. Their catalog codes were given in parenthesis. They were analytical grade materials with purity of at least 98%. *Lactobacillus plantarum* NCIMB 1193 was obtained from METU, Food Engineering Department.

## 2.2 Methods

The characterization of the raw material was done by measuring the moisture and ash content according to AOAC methods [181], and calculating the total carbohydrate portion.

In the preliminary experiments, water-bath extraction was applied to pulp obtained from juicing of the tubers and grated tubers to different sizes (2x6x40 mm, 2x2x40 mm, and 2x2x2 mm) at different temperatures (40-80°C) for different time durations (10-60 min). The procedures used in the experiments were given in Figure 9 (for juicing) and Figure 10 (for grated JAs).

For the main bulk of the experimental studies, one step extraction with water either in a shaking water-bath or in an ultrasonic bath was chosen to produce syrups, using 10g of grated JA in all experiments. Water-bath was temperature-controlled, but ultrasonic bath was not temperature-controlled. Thus, for the ultrasonic extractions done at temperatures other than room temperature, water was heated up to the desired temperature and then put into the bath and ultrasonication was applied during determined time duration. The ultrasonic bath (Model 1510 MT) has a frequency of 40 kHz, a power of 50W, and tank size of 5.5’’x6’’x 4’’ with tank capacity of 1.8L. The effect of microwaving of the tubers was also investigated by following the same procedure as in the water-bath extractions by microwaving for 1 min and cooking of the tubers by microwaving them for 20 min in a microwave oven that has a power of 1250 W, therefore applying 125W/g of sample.

Finally, yield of extraction, degree of polymerization, product profile, physical and functional properties of the syrups were determined.

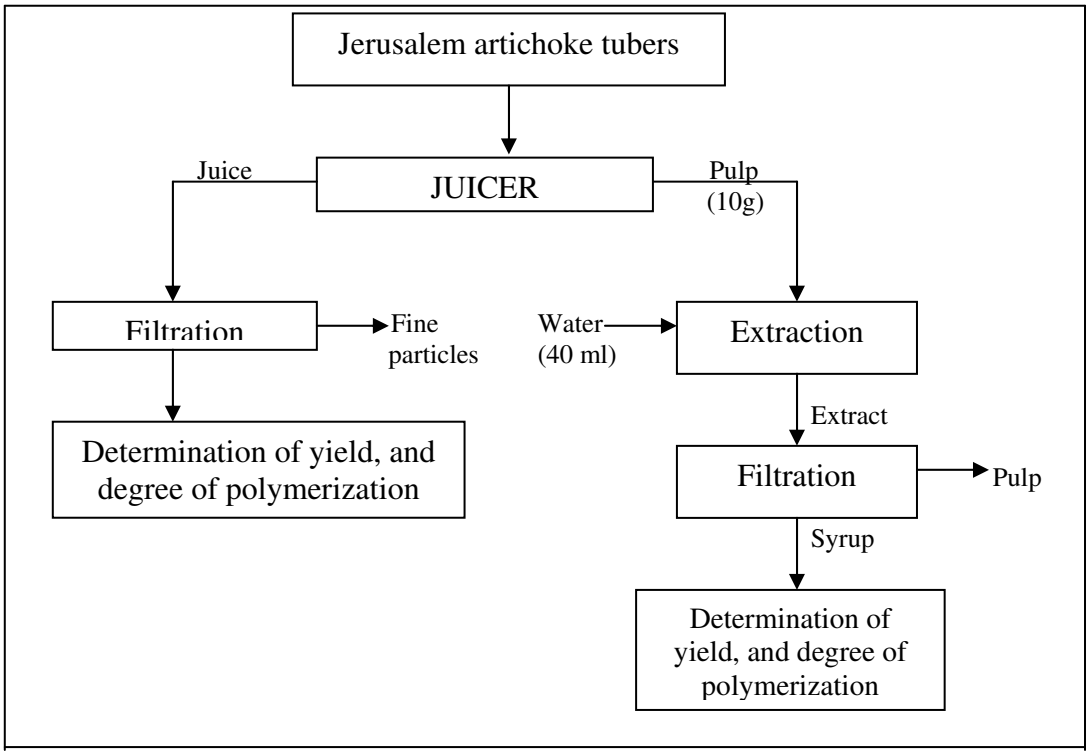


Figure 9. Experimental procedure used in the preliminary experiments done with juiced tubers.

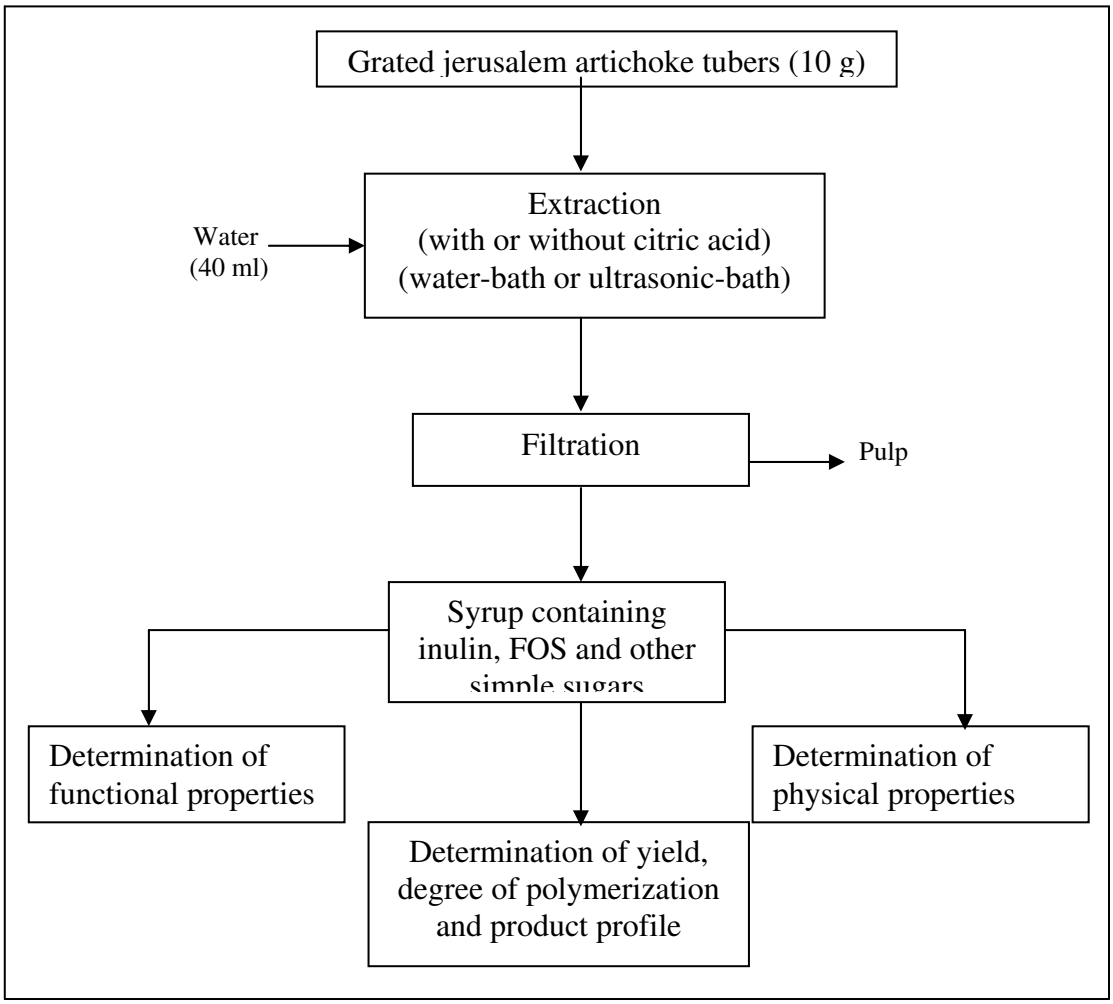


Figure 10. Experimental procedure used in the experiments done with grated tubers.

### 2.2.1 Experimental Parameters

Several parameters including extraction time and temperature, particle size, amount of solvent, harvest date and storage time of the tubers, were investigated in specified ranges represented in Table 14. Since citric acid addition was selected as another parameter. Thus, the pH of the medium was followed during and after the extraction.

Table 14. Experimental parameters used in experiments

| Method / Parameter | Conventional Extraction | Microwaving (1 & 20 min) | Ultrasonication |
|---|---|---|---|
| Grated JA (g) | 10 | 10 | 10 |
| Extraction time (min) | 10-60 | 40 | 3 & 3.5 |
| Extraction temperature (°C) | 40-80 | 60 | 20, 40 & 60 |
| Amount of solvent (ml) | 30-50 | 40 | 40 |
| Citric acid concentration (mM) | 0-39 | 0 & 26 | 0 & 26 |
| Harvest date | January-May | June 2005 & February 2006 | April |
| Storage time (day) | 0-20 | 0 & 30 | 0-20 |

### 2.2.2 Extraction Experiments

Preliminary experiments done by grating the tubers into different sizes by juicer, extracting 10g of pulp with 40ml of water, and finally determining the yield and DP in both filtrate (Figure 9).

In all of the experiments except some of the preliminary ones, jerusalem artichoke tubers were grated by using an ordinary food processor (Arçelik) to obtain 2x6x40mm (average) particle size. Simultaneously the solvent was placed in a water bath to reach the desired temperature. After that, 10g of these jerusalem artichoke particles were added into the flask containing specified amount of solvent at specific temperature. This process was done quickly because of coloring reaction due to polyphenol oxidases in the tubers. Then, flask was put into the ultrasonic-bath or shaking water-bath for extraction up to specified time. Finally, the syrups obtained by filtration were used for analysis.

## 2.3 Analyses

### 2.3.1 Dry Matter Analysis and Calculation of Yield

The extraction yield was determined by dry matter analysis; drying solutions at 105°C for 24 h in an oven (AOAC, 2000; 33.2.44, 990.20) [181]. Yields based on dry matter ($Y_{DM}$) and 100g of JA ($Y_{JA}$) obtained were defined as:

$$\text{Yield based on dry matter} = \left( \frac{\text{Dry matter in syrup (g)}}{\text{Dry matter content of JA tuber used}} \right) \times 100$$

$$\text{Yield based on 100g JA} = \left( \frac{\text{Dry matter in syrup (g)}}{\text{10g of JA}} \right) \times 100$$

The figures containing the data of $Y_{DM}$ versus investigated parameters were given in Appendix A.

### 2.3.2. Nelson-Somogyi Analysis and Calculation of DP and MU

In order to determine the degree of polymerization, reducing end determination by the Nelson-Somogyi method was used [182]. This method depends on the color reforming of reducing ends with the components used as reagent. Color changes were analyzed based on absorbances at 520 nm by Hitachi U-3200 spectrophotometer. The details of the method and calibration curve were given in Appendix B.

According to the analysis, monosaccharide units (MU), reducing ends (RE) and the degree of polymerization were defined as follows:

$$\sum MU\ (mmol) = \left[ \frac{\text{Total dry matter (mg)}}{(DP - 1) \times 162 + 180} \right] \times DP$$

$$RE\ (mmol) = \left[ \frac{\text{RE (mg G)}}{180} \right]$$

Since DP = [∑MU (mmol) / RE (mmol)];

$$DP = \left[ \frac{\text{Total dry matter in the sample (mg)}}{\text{RE in the sample (mg G)}} \times 180 - 18 \right] \times (1 / 162)$$

If the DP was not measured MU were calculated from the formula:

$$MU\ (mmol) = [Y_{DM}\ (\%) \times \sum MU \text{ in JA(mmol)}] / 100$$

### 2.3.3 HPLC Analysis

Determination of product profile was done by HPLC in the Central Laboratory of METU. In this analysis, a carbohydrate analysis column, Aminex HPX-42C (Biorad) was used with Refractive Index detector. The column temperature was 80°C, and the mobile phase was distilled water with flow rate of 0.6 ml/min. The compounds that have a degree of polymerization up to four were accurately determined in these analyses, since only the standards of these compounds (fructose, glucose, sucrose, 1-kestose and nystose) were available. The amounts of sugars with DP of 5 and 6 giving distinct peaks (as represented in a sample chromatogram in Figure 11) were estimated by using this formula:

$$A \times MW \times 10^{-6} = 47.006 \#F^2 - 112.25 \#F + 338.68$$

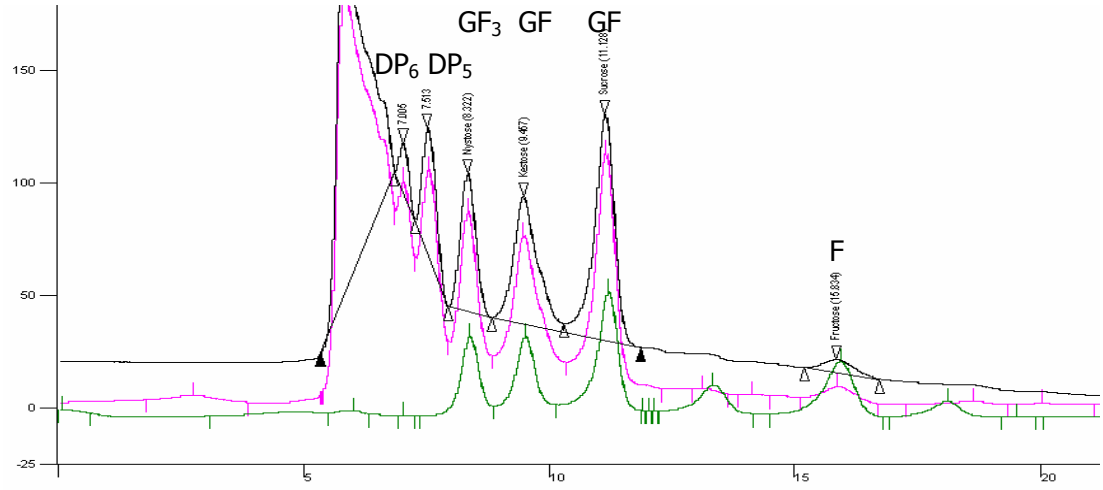


Figure 11. Sample chromatogram obtained with the water-bath extraction of March 2006-harvested, 15 day-stored JA tubers under non-acidic conditions

The formula used was obtained by searching the relation between the calibration constants calculated by area / concentration of the sugars to the number of fructose units. The raw data, sample calculations and chromatograms were given in Appendix C, D and E, respectively.

As it was stated in Chapter 1, according to the definition of inulin, both $GF_n$ and $F_m$ compounds are considered to be included. Since no standards of $F_m$ compounds are available, the peaks obtained by maltose ($G_2$) and maltotriose ($G_3$) were also investigated and compared with sucrose (GF) and 1-kestose ($GF_3$), respectively in

order to see the effect of changing glucose molecule with fructose in the compound in elution time of the peaks obtained by HPLC.

### 2.3.4 Prebiotic Property Analysis

There are several ways used in the literature to demonstrate the fermentation of fructo-oligosaccharides as stated in Chapter 1. Since FOS were not synthesized from sucrose, radiolabelling was not appropriate for this study. The method of formation of colored colonies can not be quantified the fermentation of FOS by microorganisms. Due to that reason, it was thought that prebiotic property can be analyzed by measuring the growth rates. To ensure that the growth occurs via the fermentation of FOS and not by other sugars (such as glucose, fructose, and sucrose found in syrups), three ways were possible; separation of these sugars from the syrups by chromatographic methods, consumption of these syrups by microorganism that is non-fermenter against FOS or using a control medium containing the same amounts of these sugars as a carbohydrate source for the microorganisms chosen to be studied. Due to the time limitations last choice was chosen.

Thus, in this study, the prebiotic property of the syrup obtained was analyzed by fructo-oligosaccharide fermenter bacterium, *Lactobacillus plantarum* NCIMB 1193. Microorganisms were grown on two liquid media. The first medium contained MRS basal (no carbohydrate source, only vitamins, minerals and some nucleic acids) added to which is 10ml of the syrup produced. The second medium was called as standard containing MRS basal (citrate omitted) and 10 ml of a solution at the same concentration of glucose, fructose and sucrose mixture in the syrup obtained. The composition of the MRS basal medium was given in Appendix F. In these experiments bacterium was activated by transferring them in 10ml of growth medium and incubated at 20°C overnight. This procedure was applied twice. After that, 200µl

of them were transferred into 200ml of growth media. Three replicates were used. Finally every hour two samples each of which has 1.5ml of the growth medium were analyzed in a spectrometer at 600nm. The deviations in absorbance values were $\pm 1.9.10^{-3}$. The blank was the standard media with no bacteria in it. Dilution was applied by adding 750µl blank solution into 750µl sample, if needed.

Growth curves of organisms in these media in terms of averages of the absorbances were compared directly, since only a comparison of the fermentation of the syrups produced via different methods were needed.

### 2.3.5 Physical Property Analysis

The syrup density, viscosity and color were analyzed in this part. Densities of the syrups were determined by weighting 10 ml of each solution. This procedure was applied twice and average values were used in calculations.

In order to determine whether the syrup is Newtonian or Non-Newtonian, Cone and Plate viscosimeter was used. It consists essentially of a stationary flat plate, upon which is placed a puddle of the liquid to be tested, and an inverted cone, which is lowered into the puddle until its apex just contacts the plate. The cone (8 cm in radius) was rotated at a known angular velocity Ω (10-20rad/s), and the viscosity of the fluid was determined by measuring the torque required to turn the corn. The angle between the conical and flat surfaces was kept small, about one half of a degree. At those torques, the viscosities of the syrups were calculated according to Bird et.al. [183].

Viscosities of the syrups were determined by Ostwald viscosimeter [183]. This procedure depends on measuring the time required to move the solution between the

lines explicitly determined on the viscosimeter. But in order to calculate the viscosity of the solution, the constant for the viscosimeter was determined first by using a liquid with known viscosity. For this, water was used. After calculating the constant for the viscosimeter $A_v$, the viscosities of the solutions were calculated by using the following formula:

$$\text{Viscosity} = A_v \times \rho_{sol} \times t_{sol}$$

Measurements were done twice and average values of them were used in calculations.

Two methods were used for color measurements, as cited in the literature for food analysis [184, 185]. To determine the darkness of the syrup, the absorbance values of them were measured twice at 420nm spectrophotometrically. The color evaluation of the produced syrups was performed on the basis of readings of results of instrumental measurements. The color was measured utilizing the Hunter Lab system, with direct reading of the L (Hunter luminosity), a (red intensity) and b (yellow intensity) values. No dilutions were used in these analyses. Commercial apple juice was taken as standard and the value of color difference ΔE was calculated between the color of each sample and the model, commercial apple juice, using the following formula:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$$

To determine the significancy of the results of density, viscosity, darkness, and color analysis, Student's t distribution test was applied to the data obtained from those measurements [186]. Sample calculations can be seen in Appendix D.

### 2.3.6 Reproducibility Analysis

Since the contents of jerusalem artichoke were changing with storage time, harvest date, weather and soil conditions, in order to determine the reproducibility of the experiments, two considerations were taken into account for water-bath extractions. Firstly, the results of extracts obtained by using jerusalem artichokes (that have the same harvest date, taken from the same field, and stored in a refrigerator during the same durations) were compared. The content and the distribution of degree of polymerization in tubers are affected by variations in sunlight or rain conditions, or nutrient within the same field. Thus those analyses may not be enough, because the observed differences may have resulted from factors mentioned above. So, water-bath and ultrasonic-bath extractions were also applied to the jerusalem artichoke tubers five times for each in the same day to eliminate the effect of weather and soil conditions. In these experiments 40ml water containing 26mM citric acid was used as a solvent. For ultrasonic extractions only the last procedure was applied. Dry matter, Nelson-Somogyi, and HPLC analysis were applied to the extracts, and the results were compared. The data of these experiments were given in Appendix G, and sample calculations can be seen in Appendix D.

# CHAPTER 3

# RESULTS AND DISCUSSION

The raw material was characterized as 78.9% moisture and 1.3% ash content. Thus total carbohydrate portion was calculated as 19.8%.

The syrup that has the high yield, low degree of polymerization with high ratio of functional (DP 3-6) to waste sugars (DP 1-2) had been defined as the best syrup for those conditions. The sugars that have DP 1-2 were regarded as waste according to prebiotic point of view, since these sugars will be digested and will not reach the colon, thus will not metabolized by the probiotic microorganisms, but functional sugars with DP 3-6 can be consumed only by the bacteria in the colon. Since the amounts of sugars that have a DP 5 and 6 were estimated, the ratio DP [(3-4)/ (1-2)] was the accurately measured ratio of functional sugars to waste sugars. These criteria were applied both based on the extract obtained and the JA sample used in the experiments. In addition, in HPLC analysis, it was found that the retention time and area of the peaks of sucrose (GF) and maltose ($G_2$) were found as the same, those of maltotriose ($G_3$) and 1-kestose ($GF_2$) were found different. Thus, it was concluded that HPLC may or may not measured all $F_m$ compounds found in the extracts since the peaks of $GF_2$ and $F_3$, $GF_3$ and $F_4$, etc. may also be the same. The peaks of GF and $F_2$ may also be the same indicating more functional content in the syrups than measured since the amounts of sugars defined as sucrose may be $F_2$.

The significantly different results according to reproducibility analysis and student's t distribution test were represented with different letters in the corresponding figure. It did not mean that the values having same letters in different figures were the same, unless otherwise stated.

## 3.1 Reproducibility

### 3.1.1 Water-bath Experiments

Because of the changing content of the tubers, in order to determine the reproducibility of the experiments two procedures were used. In both, jerusalem artichoke tubers of the same harvest date, taken from the same field, and stored in a refrigerator for the same durations were used. In the first method, the differences between the yields and degree of polymerization values in the years 2005 and 2006 were found as 4.9% and 5.9% for fresh-NA conditions, 0.9% and 0.9% for 10 day-stored-NA conditions, 0.9% and 1.7% for 10 day-stored-A conditions. But in the second procedure, extractions were done five times to eliminate the effects of weather and soil conditions. Dry matter, Nelson-Somogyi, and HPLC analysis were performed on the extracts. Sample calculations can be seen in Appendix D. As can be seen from Appendix F, in the second procedure 0.9% difference in yields obtained from dry-matter analysis, 1.3% difference in DP obtained from Nelson-Somogyi analysis. In investigation of the data, average values (1.6% in yield, 2.1% in DP) were taken into consideration. The highest standard deviation in repeated HPLC analysis was ±0.0023 thus average values were used. The reproducibility results were as follows; 0.59% difference between the highest and lowest values in DP 1-2, 0.73% in DP 3-4, and 0.82% in DP 3-6 were observed based on extracted amount of total monosaccharide units, thus they were neglected. By doing similar calculations based on jerusalem artichoke sample used in extractions, 0.48% difference in DP 1-2, 0.39% difference in DP 3-4, and 0.54% difference in DP 3-6 were obtained. In investigation of the data, respective reproducibility value was taken into consideration.

### 3.1.2 Ultrasonic-bath Experiments

To determine the reproducibility of ultrasonic-bath experiments, jerusalem artichoke tubers were extracted with 40 ml of water under acidic conditions by following the same procedure described in Methods section in Chapter 2. These experiments were done five times in the same day, due to the same reasoning described above. Dry matter, Nelson-Somogyi, and HPLC analysis were performed to the extracts. As can be seen from Appendix F, 2.4% difference in yields obtained from dry-matter analysis, 3% difference in DP obtained from Nelson-Somogyi analysis. The highest standard deviation in HPLC analysis were ±0.0064, thus average values were used. The results of reproducibility were as follows; 1.52% difference between the highest and lowest values in DP 1-2, 1.82% in DP 3-4, and 2.62% in DP 3-6 were observed based on extracted amounts, thus these were neglected again. By doing similar calculations based on jerusalem artichoke sample used in extractions, 0.47% difference in DP 1-2, 0.7% difference in DP 3-4, and 0.84% difference in DP 3-6 were obtained. It was concluded that the most reliable analysis was HPLC as in the case of water-bath extractions. When ultrasonic-bath analysis was compared with water-bath, it could be concluded that ultrasonication was less reproducible than water-bath.

## 3.2 Preliminary Experiments

In this part of the experiments, experimental procedure described in Figure 9 in Chapter 2 was used, and extraction of the pulp was performed during different extraction times. The yield values were calculated by applying material balance to

the system composed of pulp and juice. The theoretical yield was taken as 75% of the dry matter of JA sample used [144]. The results were shown in Figure 12. Highest yield (14.4g) was obtained via 40 min extraction at 60°C. No significant change was observed for longer times. The contribution of juice and extraction of pulp to the yield values were given in Figure 13. For 40 min, these values were 78% and 22% for juice and extract, respectively. Thus, extractions in preliminary experiments were done during 40 min.

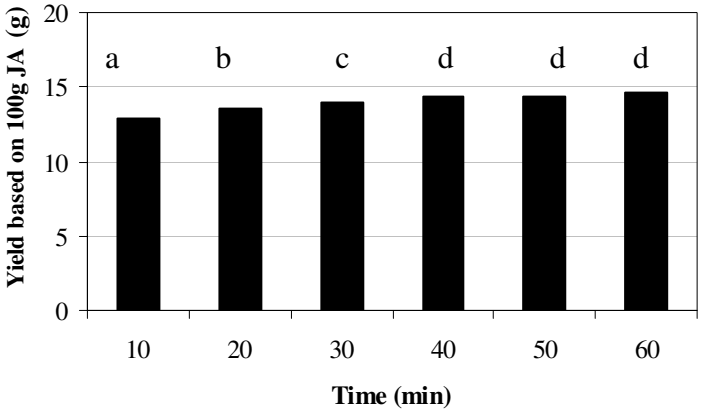


Figure 12. The effect of extraction time on yield

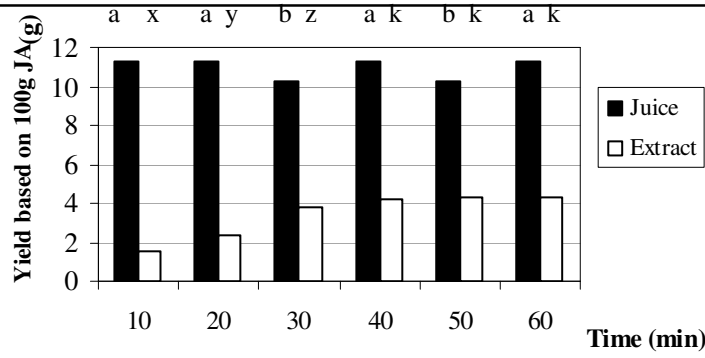


Figure 13. The contribution of extraction of JA pulps with respect to time to the yield

To determine the most suitable temperature, pulps were extracted at different temperatures, and the results were given in Figure 14. The highest yield (15.3g) was obtained via 40 min extraction at 60°C.

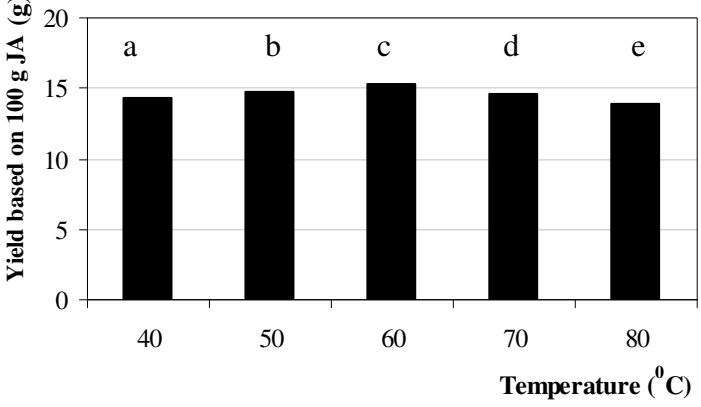

Figure 14. The effect of temperature on extraction yield of extracts obtained from JA pulps

The effect of temperature on degree of polymerization of the extracts of pulps was shown in Figure 15. In that, the degrees of polymerization of the juices were also given just for comparison. Lowest degree of polymerization (6.3) was obtained by extracting the pulps at 60°C. Thus, the preliminary extraction experiments were done at this temperature. As it can be seen from Figures 14 and 15, the degree of polymerization decreases as the yield increases, probably because shorter compounds are more easily extracted from the tubers.

The observation of higher DP and lower yield obtained in extractions at 70 and 80°C (Figure 14 and 15) may result from the inactivation of the enzyme hydrolyzing inulin in JA samples.

As can be seen from Figure 15, the degree of polymerization of the juice (nearly 33) was very high, thus it was concluded that juicing was not a good choice for production of these syrups. The desired range of DP can be reached by extracting the tubers after grating. So, to determine the effect of particle size, jerusalem artichoke tubers were grated into different sizes as described in methods section and extracted with 40 ml of water at 60°C for 40 min as described in Figure 10 in Chapter 2.

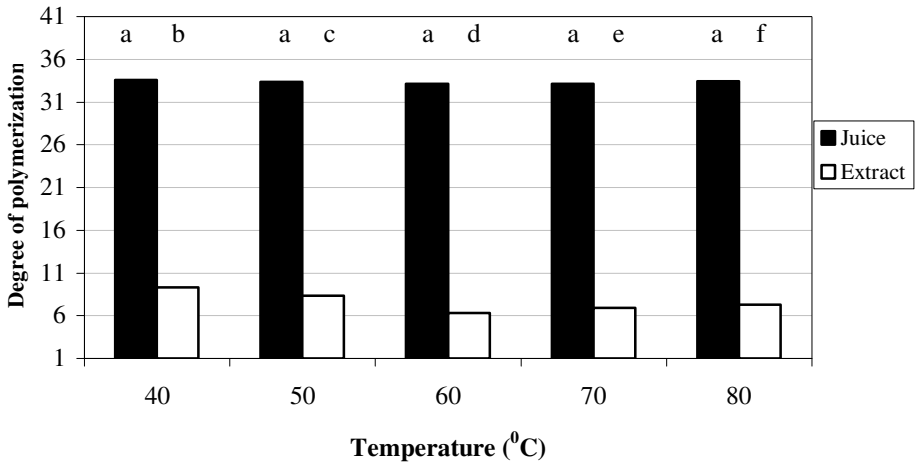


Figure 15. The effect of temperature on degree of polymerization of extracts obtained from JA pulps

The effect of particle size on yield and DP of extracts were demonstrated in Figure 16. As it is expected, reducing the particle size increased the extraction yield nearly 27% (from 12.1g to 15.3g). The effect of diffusion in a particle was determined.

Also, reduction in the particle size caused a decrease in degree of polymerization of the extracts, because inulin hydrolysis may be induced by grating the tubers. Although, the smallest degree of polymerization was obtained with cubic jerusalem

artichokes, considering the industrial application, jerusalem artichokes were grated in rectangular prism (2x6x40 mm, average) in experiments of the study.

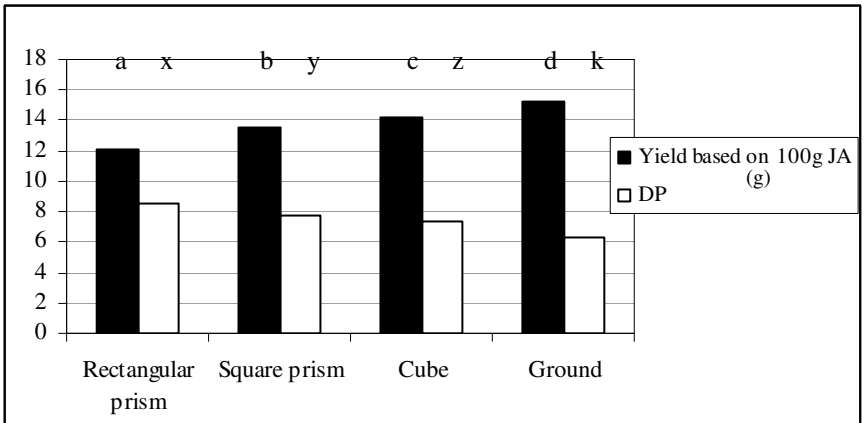


Figure 16. The effect of particle size on yield and DP of extracts obtained from grated tubers

### 3.3 Extraction Time

In order to verify the extraction time found in preliminary experiments, 10g samples of grated February 2005-harvested fresh jerusalem artichoke tubers were extracted with 40ml of water at 60°C. As a result of the experiments, syrup yield and a degree of polymerization (Figure 17) were obtained as 15.8g and 6.6 via 40 min extraction. For longer times no significant change was observed in yield or in degree of polymerization. Thus, for ongoing experiments 40-min extraction was used. The difference between the yield values obtained by the extractions at 60°C for 40 min (14.4g represented in Figure 12, 15.3g in Figure 14, and 15.8g in Figure 17) may resulted from different inulin content in JA samples due to whether and soil

conditions of different fields, and also the effect of harvest date and storage time of the tubers.

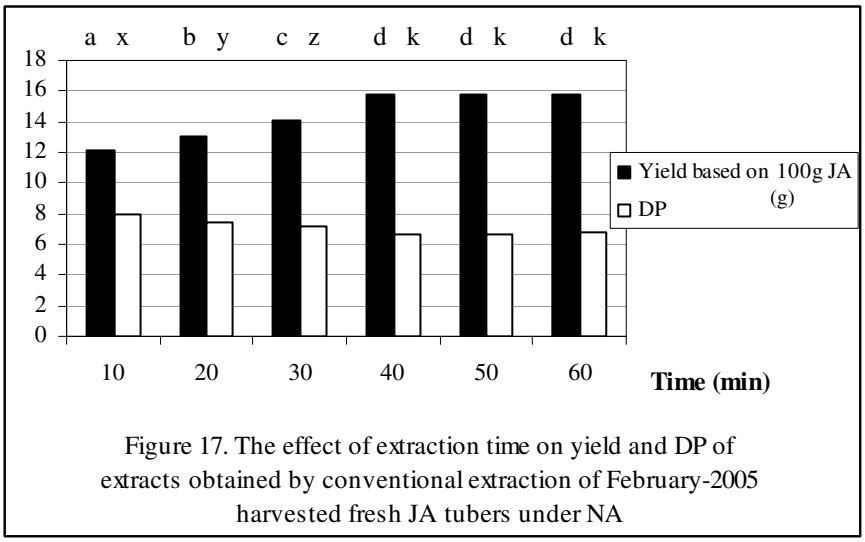


Figure 17. The effect of extraction time on yield and DP of extracts obtained by conventional extraction of February-2005 harvested fresh JA tubers under NA

### 3.4 Extraction Temperature

To verify the most suitable temperature, October 2004-harvested fresh jerusalem artichoke tubers were extracted with 40 ml of water during 40 min. The highest yield (nearly 15g) and lowest degree of polymerization (less than 7), (Figure 18) was obtained at 60°C. The trend of decrease in DP with increase in yield observed in extraction of pulp in preliminary experiments was obtained again in the extraction of grated tubers.

It was reported in the literature that the rate of extraction from JA tubers increases with increasing temperature up to about 60°C, but no differences observed with higher temperatures [147]. But, in this research decrease in yield and increase in degree of polymerization were observed at 70°C and 80°C. It can be explained by the

inactivation of the native inulinase enzyme, since these temperatures are so high according to the optimum temperature of 40 and 56°C stated in the literature [142, 143]. It may also result from the change that decreases the permeability of the tissue. In addition, it was stated in the literature that 56°C prevent contamination, thus in following experiments 60°C was chosen.

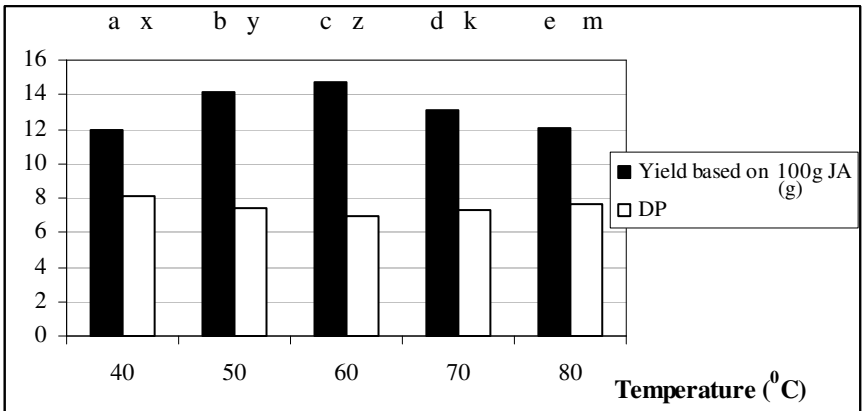


Figure 18. The effect of temperature on yield and DP of extracts obtained by conventional extraction of October-2004 harvested fresh JA tubers under NA

## 3.5 Amount of Solvent

The results of the extracts of December 2004-harvested fresh jerusalem artichoke tubers were given in Figure 19. The extractions were done at 60°C for 40min. Highest yield (12.7g) was obtained by extracting with 40 ml of water. The decrease in yield obtained by using 45 and 50 ml of water may result from the less inulin content of the JA sample used, which may due to high nitrogen fertilization of that portion of the field that reduced the accumulation of inulin in JA tubers [140]. Thus solid to solvent ratio was chosen as ¼ for following experiments, as has also been suggested by Wenling et.al [103].

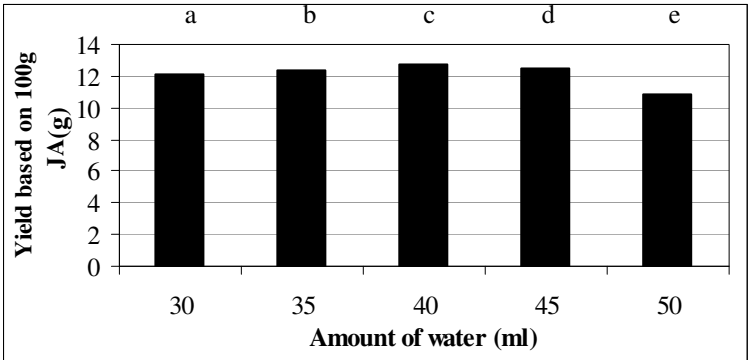


Figure 19. The effect of the amount of solvent on yield of extracts obtained by conventional extraction of December-2004 harvested fresh JA tubers under NA

## 3.6 Peeling

To observe the effect of peeling, February 2005-harvested, 10 day-stored tubers were extracted with 40 ml of water at 60°C for 40 min. Obtained yield values were 17g and 13g, and degree of polymerization values were 7 and 7.9 for extracts obtained with whole and peeled tubers, respectively. Nearly 31% increase in yield, and 13% decrease in degree of polymerization was obtained with whole tubers. Thus, it can be said that the native inulinase enzyme is in or near to the shell of the tubers. So, for the production of functional syrups, tubers should be used as a whole.

In the literature, it was stated that the non-soluble material (mostly the skin of tubers) may cause some problems of pumping if the JA tubers were cooked [141]. Thus, separation processes such as filtration should be applied to remove the cellulosic material. There is no information about the location of the enzyme in the tubers in the literature. The only information was that it is in the vacuole of the cells [135].

### 3.7 Citric Acid Addition, Harvest Date & Storage Time

In the research of Ziyan and Pekyardımcı [145], several inhibitors including citric acid were studied. Although the amount of inhibitor was not stated, they found 50% and 89 % activity remaining after addition of citric acid for skin and flesh PPO. In this study, citric acid was added into the water in different concentrations in order to inactivate the PPOs found in the tubers. Same experimental procedure was applied to the June 2004-, and February 2005-harvested 10-day-stored tubers. In both of the years, ignoring no acid addition, the highest yields (13.1g in 2004, and 16.2g in 2005 as represented in Figure 20) were obtained with 26 mM citric acid-added extracts. Considering reproducibility of Nelson-Somogyi analysis, almost no significant change was observed on degree of polymerization of the 26 and 39mM citric acid-added extracts in the year 2005, whereas the smallest degree of polymerization was obtained with 39 mM citric acid added extracts in the year 2004 (Figure 21). The lowest DP values obtained were 7.9 and 6.5 in the years of 2004 and 2005, respectively. The differences between the yields (nearly 24%) and DP of syrups in these years may result from the varied inulin content of JA samples due to differences in climate conditions during growth. Since higher yield and lower DP obtained in the year 2005, it was concluded that this year was better than 2004. To obtain high yield and low degree of polymerization, and considering the bitter flavor of 39 mM acid added extracts, 26 mM citric acid addition was chosen for all acid-added experiments.

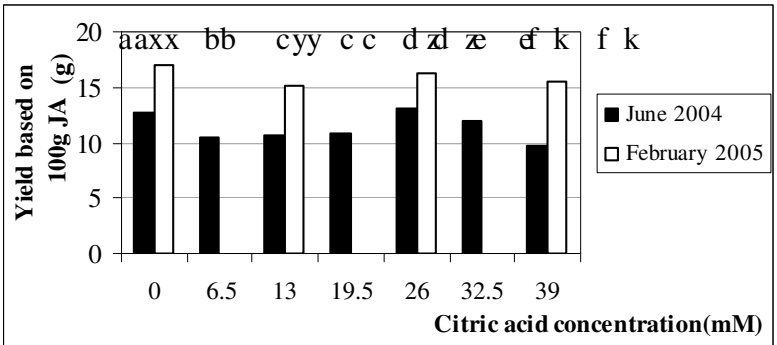


Figure 20. The effect of citric acid addition on yield of extracts obtained by conventional extraction of June 2004 and Febnruary 2005 harvested, 10 day-stored JA tubers

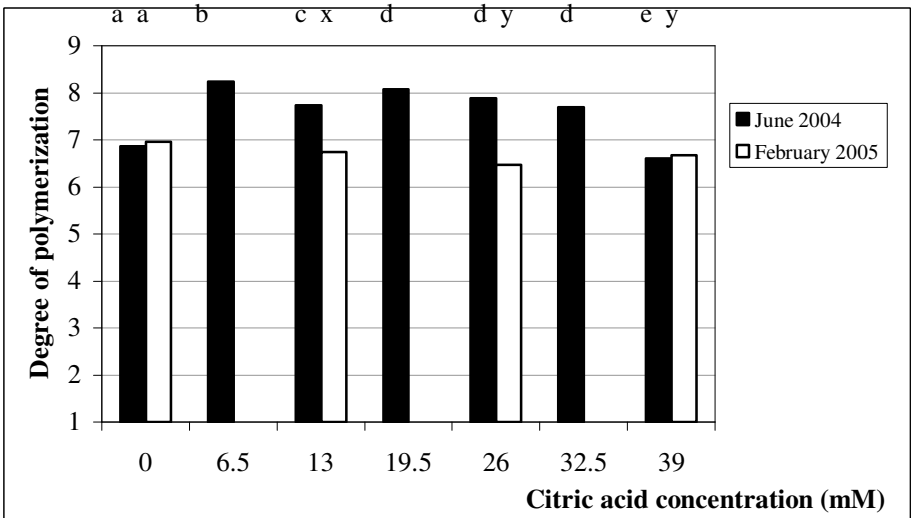


Figure 21. The effect of citric acid addition on degree of polymerization of extracts obtained by conventional extraction of June 2004 and February 2005-harvested 10 day-stored JA tubers

pH of all acid-added experiments were followed during and after the extraction. As can be seen from Figure 22 that the medium was found to behave as a buffer during extraction. The pH of 26 mM citric acid added extracts was found to be 3.8.

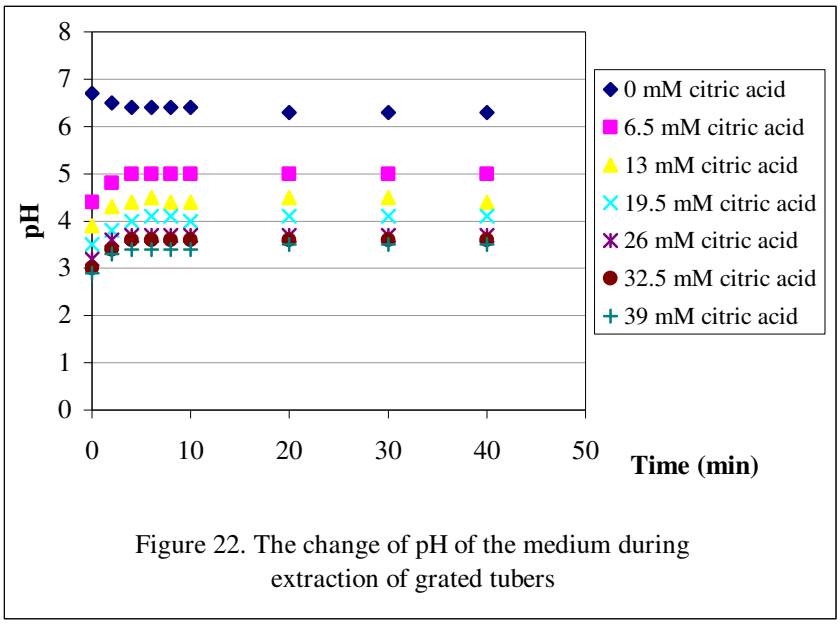


Figure 22. The change of pH of the medium during extraction of grated tubers

Following pH of extracts up to 90 minutes after the completion of extraction, no change in degree of polymerization was observed in extracts obtained with or without acid addition (Figure 23).

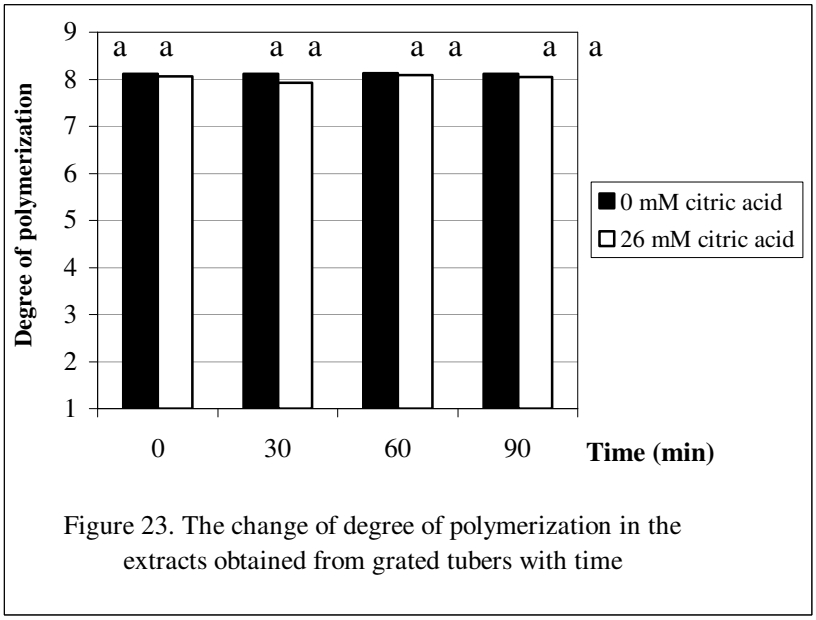


Figure 23. The change of degree of polymerization in the extracts obtained from grated tubers with time

As a result of the experiments presented up to here, it was found that juicing was not applicable for production of these syrups since the DP of the juice was found as 33 that were so higher than the desired range. Thus following experiments were done by extracting the tubers in rectangular prism. The optimum conditions for the extractions were found as 60°C, 40 min, 40 ml of water containing 26mM citric acid and whole tubers usage. Under those conditions, depending on the harvest date, storage time, weather and soil conditions, the syrups having 12g to 16g yield based on 100g of JA tuber, and a DP of 6 to 7 were obtained. By comparing the results with the similar studies in the literature summarized in Table 15, it was observed that nearly the same yields were obtained with extractions with reduced temperature and time.

Table 15. Summarized optimum conditions for extractions stated in similar studies in the literature

| **Researcher** | **Pre-extraction** | **Solvent** | **Time** | **pH** | **Temp ($^0$C)** | **$Y_{DM}$ (%)** |
|---|---|---|---|---|---|---|
| Conti [149] | No | water | 1h | 4.4 | 80 | 80 |
| Fleming et al. [147] | No | water (0.1%$SO_2$) | 30 min | 1-2 | 70 | 95 |
| D'Egidio et al. [132] | 96$^0$C ethanol | water | 2 h | - | 105 | 90 |
| Wenling et. al. [103] | No | water | 40 min | - | 100 | 75 |

The pH of the syrups was found as 3.8 which were lower than the study of Conti, and higher than the study of Flemming and coworkers. Acid addition aiming color reduction may also prevent contamination as it was aimed also by Flemming. The pH of the syrups was also lower than the optimum pH of the native inulinase enzyme stated in the literature as 5.1 and 6.5 [142, 143]. Since the stated values have a wide

range, the enzyme may be active at that pH, too, or the lower pH contributes to hydrolysis as indicated by the high yield in Flemming et.al. study [147] at pH 1-2.

As it is mentioned before, the inulin metabolism of the tubers, thus the degree of polymerization depends on harvest date, storage time and weather conditions [132, 140]. The effect of harvest date can also be seen by comparing the results of the experiments done in the years 2004 and 2005 given in the Figures 17 to 21, or by following the data given in Appendix C. These results were summarized in Table 16.

Table 16. Summary of obtained yield and degree of polymerization values in the years 2004 and 2005

| | June 2004-10 day-stored-NA | June 2004-10 day-stored-A | October 2004-fresh-NA | December 2004-fresh-NA | February 2005-fresh-NA | February 2005-10 day-stored-NA | February 2005-10 day-stored-A |
|---|---|---|---|---|---|---|---|
| $Y_{DM}$ | 12.8 | 13.1 | 14.7 | 12.7 | 15.8 | 17.1 | 17.2 |
| DP | 6.9 | 6.9 | 6.9 | NM | 6.6 | 7.0 | 6.5 |

NM: Not measured

The values in Table 16 indicate that February was the most suitable harvest date to obtain highest yields with lowest degree of polymerization. Highest total carbohydrates were obtained in the study of Conti et.al. [149]. Also in the study of Schorr-Galindo [139] the least value of the ratio of F/G was obtained with February-harvested tubers, and the highest value was obtained with September-harvested tubers indicating the lowest DP in February as it was observed in our study and the highest DP in September.

Acid addition did not change the yield significantly. But acidic hydrolysis observed in February-harvested tubers significantly, probably due to more pronounced effect of acid on the lower average DP in JA tubers. But the decrease in DP was not up to simple sugars as in the Flemming and co-workers' study [147]. This may resulted

from the amount of acid added, since the aim of that study was to produce fructose syrups. Isomerization may be another factor for this difference.

To determine the variation of yield and degree of polymerization of the extracts with harvest date and storage time, the experiments were done by following the same procedure to the fresh tubers in February 2005, and to fresh to 20 day-stored tubers from January to May of 2006, in the absence of citric acid; from January to May of 2006 in the presence of citric acid.

The results of yields and degree of polymerization of the extracts were given Figures 24 and 25, respectively. The highest yield (16-18g in 2005 and 15-17g in 2006) and lowest degree of polymerization (6.6-6.2 in 2005 and 7.4-6.4 in 2006) of the extracts were obtained in mid-February of these years, in all storage times, as they were represented as pink letters. The differences between yields and degrees of polymerization of these years may result from the weather conditions and soil parameters, and also it was concluded that the year 2005 was a better year than 2006, since higher yield and lower DP was obtained. It should also be mentioned that, the same relationship, decreasing degree of polymerization with increasing yield, was again observed. Small differences in yields of January 2006- and February-2006 harvested 15 and 20 day-stored samples were observed since their harvest dates were so close to each other, and the effect of harvest date may be balanced with the effect of storage time during those storage times.

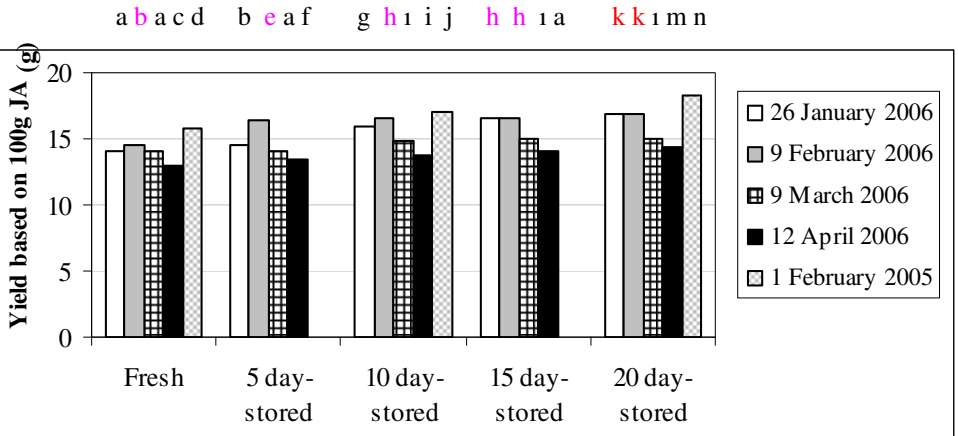


Figure 24. The effect of harvest date and storage time on yield of extracts obtained by conventional extraction in years 2005 &2006 under NA

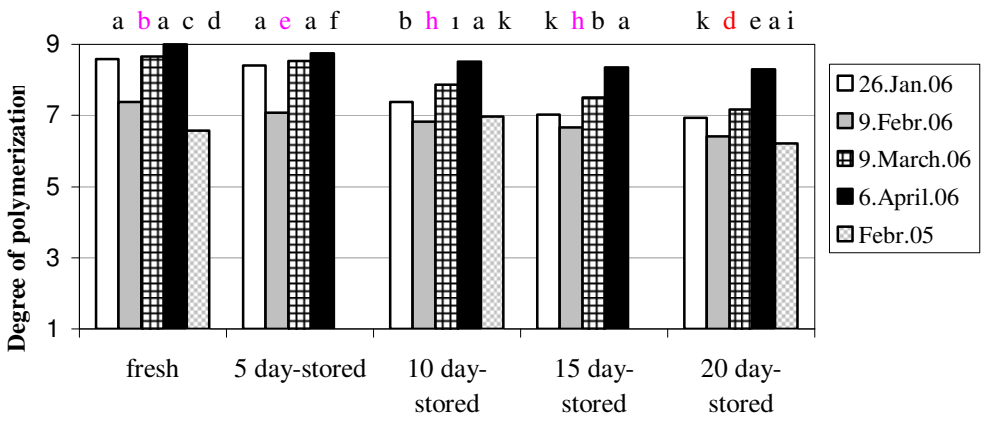


Figure 25. The effect of harvest date and storage time on degree of polymerization of extracts obtained by conventional extraction in years 2005 & 2006

By comparing the pink coded results, that belonged to the most suitable harvest date, generally as the storage time increased the yield of the extracts were increased, and the degree of polymerization were decreased, since the required energy for

respiration during storage was supplied by the inulin breakdown. The differences in between 10 and 15 day stored samples were found insignificant. This may be due to higher DP of JA sample used in the experiments because of different soil conditions. Thus, although inulin was hydrolyzed during storage as observed in other cases, the effect of the extent of hydrolysis on DP can not reach the others, since the time for complete hydrolysis dependent upon the DP of polyfructans as stated in literature [141].

According to the results obtained with 40 ml water containing 26mM citric acid at 60°C for 40 min extraction experiments done in the year 2006 (Figure 26 and 27), the same relationship between the degree of polymerization and the extraction yield was obtained. According to the reproducibility data, highest yield (15-17g) and lowest degree of polymerization (7.1-6.2) were obtained with mid-February harvested tubers, in all storage times, as again coded with pink letters. Small differences in yields of January 06- and February-06 harvested 15 and 20 day-stored samples were observed because of the same reasoning. The highest yield of 17g (Figure 26) and the lowest degree of polymerization of 6.24 (Figure 27) was obtained with February 2006-harvested 20 day-stored JA samples, coded with red letters, and thus the same conclusions can be made as in the case of non-acidic extractions.

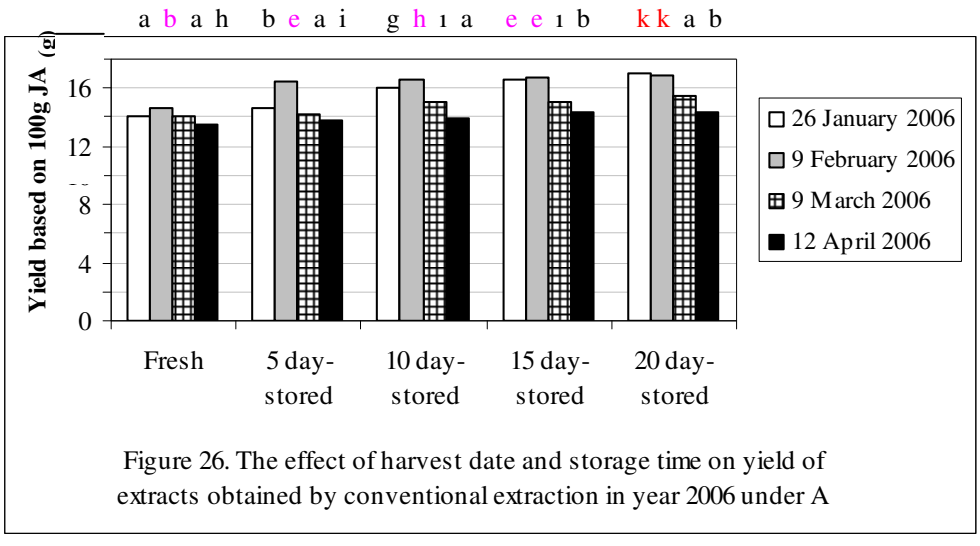


Figure 26. The effect of harvest date and storage time on yield of extracts obtained by conventional extraction in year 2006 under A

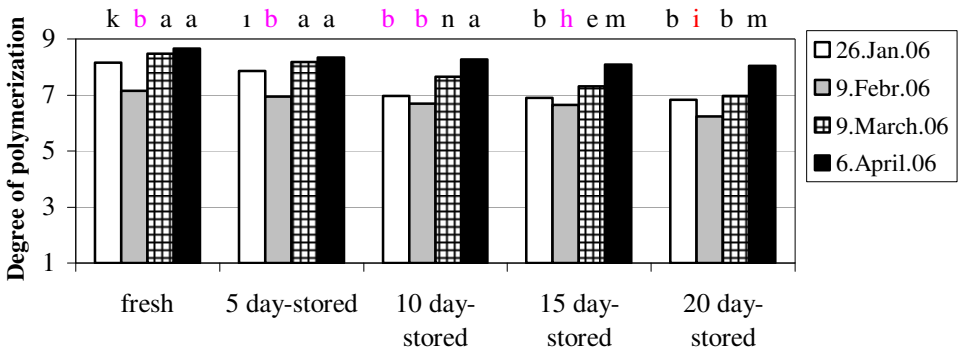


Figure 27. The effect of harvest date and storage time on degree of polymerization of extracts obtained by conventional extraction in the year 2006 under A

By comparing the results presented with the pink coded letters in Figures 24 and 25 with 26 and 27, under non-acidic and acidic conditions, it was observed that acid addition did not increase the yield, significantly, while reducing the DP under some cases depending probably on the inulin content and DP in the tubers.

The extracts obtained in experiments done in the year 2006 were analyzed by HPLC. Since the most suitable harvest date was found as February, the change of product profile of the extracts obtained in this month with storage time were represented in Figure 28 for non-acidic, and Figure 29 for acidic conditions. The amounts of waste and functional sugars were found fluctuating due to the combined effects of storage time, weather and soil conditions, and also different levels of acidic hydrolysis. Both under non-acidic and acidic conditions, 20 day storage produced the highest amount of functional sugars and total monosaccharide units extracted, and the lowest amount of waste. Additionally, acid addition increased the production of sugars investigated, but higher increase in the amounts of functional sugars than waste was observed.

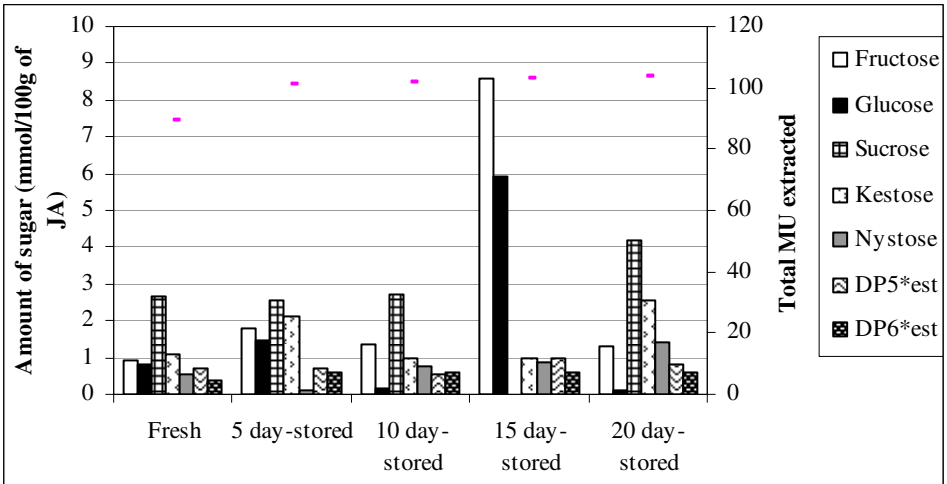


Figure 28. The effect of storage time on product profile of extracts obtained by conventional extraction of February 2006-harvested JA under NA

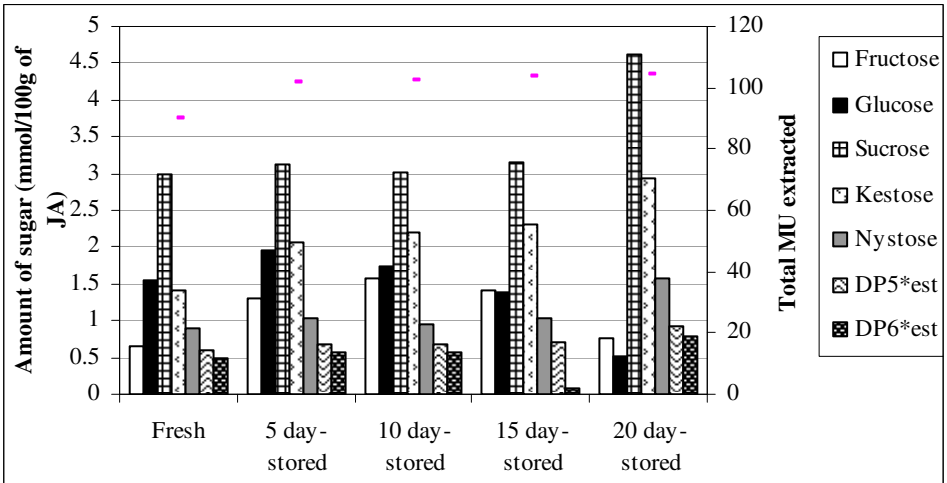


Figure 29. The effect of storage time on product profile of extracts obtained by conventional extraction of February 2006-harvested JA under A

As can be seen from Figures 30 and 31, the functionality of the syrups were found to be fluctuating with storage between 1.6 to 2 in February- and April harvested tubers,

while 1.2 to 1.4 in March-harvested ones under non-acidic conditions; 1.7 to 2.3 for February and 1.8 to 2 for April-harvested under acidic conditions. These fluctuations may have resulted from different average DP of inulin in JA samples due to soil and weather conditions, as much as different inulin hydrolysis due to requirement of different energy need for respiration during storage time; it might have to make much more respiration since its place may be the closest to the wall of the refrigerator. Comparing the results, it was found that acid addition increased the functionality of the syrups due to acidic hydrolysis. The most functional syrups were obtained with mid-February harvested, 20 day-stored tubers under both non-acidic and acidic conditions.

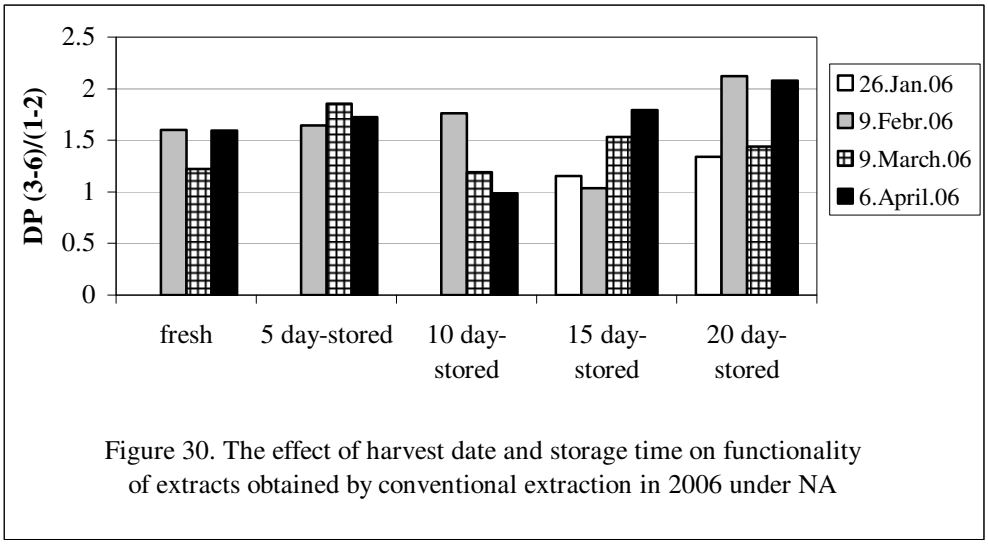


Figure 30. The effect of harvest date and storage time on functionality of extracts obtained by conventional extraction in 2006 under NA

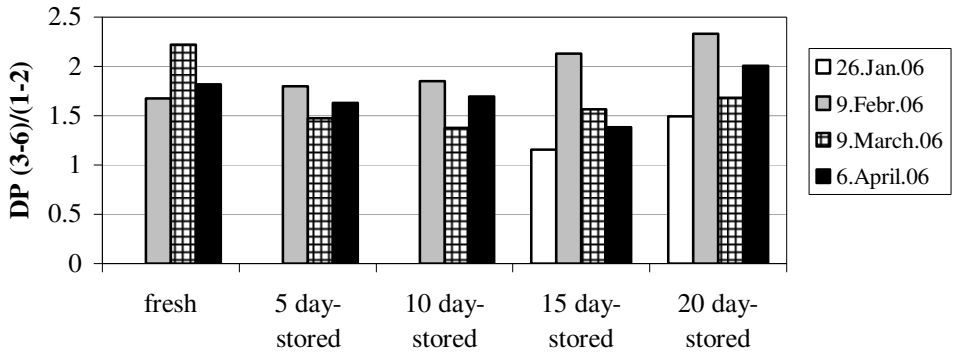


Figure 31. The effect of harvest date and storage time on functionality of extracts obtained by conventional extraction in 2006 under A

The increase in the ratio of DP (3-4)/ (1-2) was also observed in the extracts of this month in all storage times, represented in Table 17. Highest value (1.44) was obtained with 20 day-stored JA tubers under acidic conditions.

Table 17. The values of DP (3-4)/(1-2) obtained from extraction of February 2006-harvested JA tubers

| | NA | A |
|---|---|---|
| Fresh tubers | 0.75 | 0.95 |
| 5 day-stored tubers | 0.81 | 1.09 |
| 10 day-stored tubers | 0.84 | 1.11 |
| 15 day-stored tubers | 0.44 | 1.21 |
| 20 day-stored tubers | 1.36 | 1.44 |

To compare the effects of storage after harvest and keeping in the soil for the same length of time, the results of yield, DP and functionality of the syrups obtained with the extraction of 26$^{th}$ of January 2006-harvested 15 day-stored tubers under non-acidic conditions were compared with those obtained with 9$^{th}$ of February 2006-harvested fresh tubers. It was observed that although storing in a refrigerator resulted

higher yield (16.5g), lower DP (7), functionality was also lowered (1.15). The corresponding values for 9th of February 2006-harvested fresh tubers were 14.5g, 7.4, and 1.6. Thus storing in the soil up to mid-February was a better alternative to storing in the refrigerator under non-acidic conditions, since lower DP resulted from increase in the amounts of waste sugars. The observation can be explained by capability of going on taking needed nutrients from the soil.

Comparing the results of yield, DP and functionality of the syrups obtained with 26th of January 2006-harvested 15 day-stored and 9th of February 2006-harvested fresh JA tubers, same trend (higher yield, lower DP, and lower functionality obtained via storing in a refrigerator) was also observed under acidic conditions. Thus it was concluded that storing under the soil was found better under both non-acidic and acidic conditions.

Since, no significant changes (with probability level of 95%) in physical properties of tubers that have different harvest date and storage time were observed according to the Student's t distribution analysis, the average values were 0.97±0.11 g/ml, 1.03±0.09 cp, 1.89±0.4, and 42.3±2.5 for density, viscosity, darkness and color of the syrups obtained under non-acidic conditions, respectively.

For acidic conditions, the average values of the physical properties of the syrups were found 0.98±0.1 g/ml, 1.1±0.07 cp, 0.38±0.02, and 13.3±0.7 for density, viscosity, darkness, and color of the syrups, respectively. No significant change in physical properties of tubers that have different harvest date and storage time were observed, as it was observed under non-acidic conditions. It was found that acid addition decreased the color and darkness of the syrups nearly 70 and 80%, respectively, but did not affect the density and viscosity of the syrups produced.

As a result of water bath extractions, the best syrup that have highest yield and functionality, and lowest degree of polymerization was obtained with the extraction of mid-February 06-harvested 20-day stored whole jerusalem artichoke samples with 40 ml of water at 60°C for 40 min, under both non-acidic and acidic conditions. The

yield, the degree of polymerization, and the functionality values of the syrups were found as 17g, 6.4, and 2.12 for non-acidic condition, and 17g, 6.24, and 2.33 for acidic conditions. The syrup obtained under acidic conditions was found as the best since it had the desired criteria in both yield, DP, functionality and also physical properties. Obtained values, sample calculations in all of the experiments were given in Appendix C, and D, respectively.

The percentages of waste, detected and functional sugars based on extract and on JA sample were given in Figure 32 for non-acidic condition and Figure 33 for acidic condition. As can be seen the values based on JA sample was lower than those based on extract because of the yield of extraction. As a result of the experiments, 17% and 20% of functional sugar content of the JA sample was extracted under non-acidic and acidic conditions, respectively. By comparing the functional sugar content of the extracts obtained in non-acidic (17%) and acidic conditions (20%), it was concluded that acid addition increased the functionality of the syrup produced by using February-harvested JAs, as it is mentioned before.

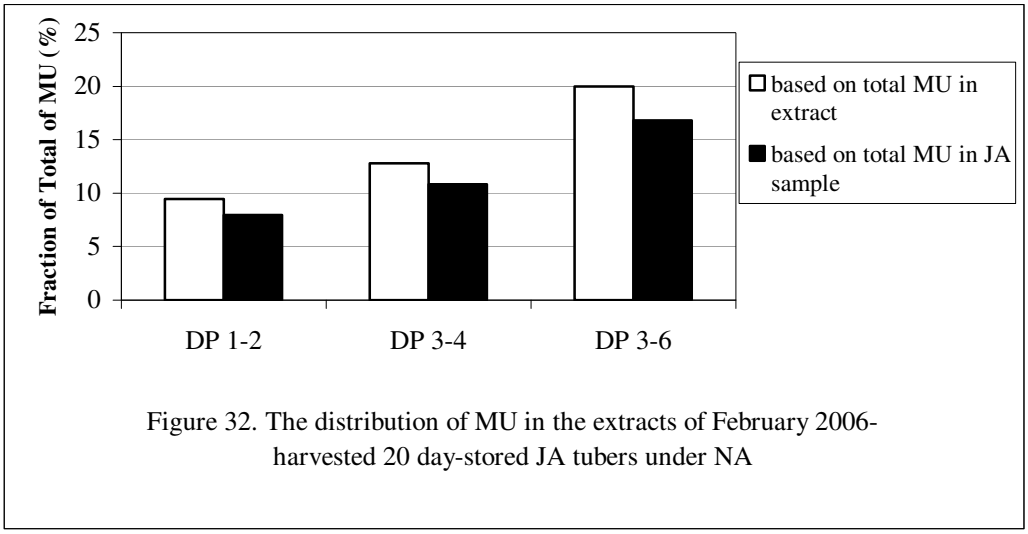


Figure 32. The distribution of MU in the extracts of February 2006-harvested 20 day-stored JA tubers under NA

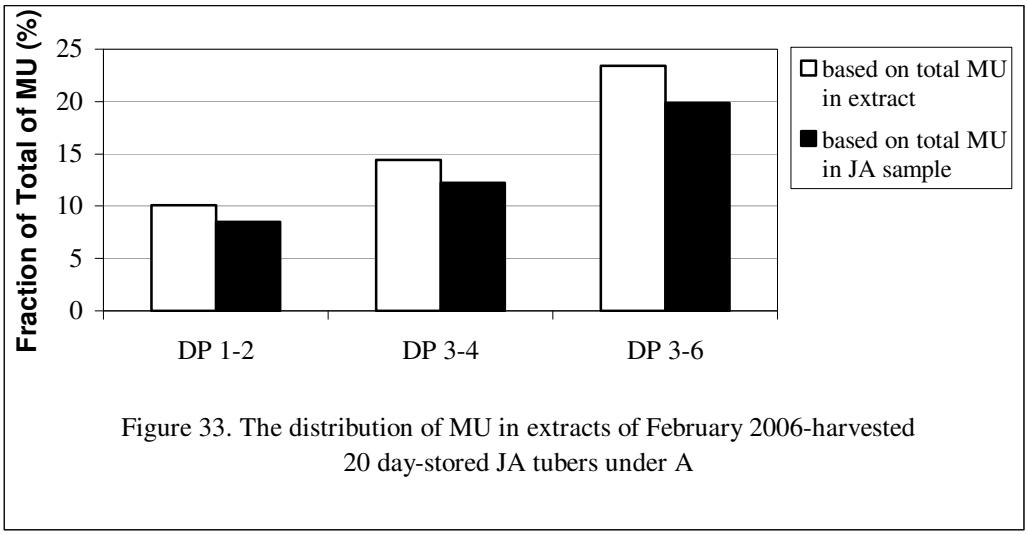


Figure 33. The distribution of MU in extracts of February 2006-harvested 20 day-stored JA tubers under A

When the amount of sugars detected or estimated by HPLC that have reducing ends (glucose, fructose, kestose, nystose, DP5*est, and DP6*est) were compared with reducing sugars detected by Nelson-Somogyi method the value obtained by HPLC was found lower (0.67 mmol under NA, 0.75 mmol under A) than the one obtained by Nelson-Somogyi Method (1.6 mmol under NA, 1.65 mmol under A). Because in HPLC the sugars that have a degree of polymerization less than five had been analyzed only, and those have only one reducing end, if any, while in Nelson-Somogyi all reducing ends in the extract was determined.

Prebiotic property of the best syrup obtained in the conventional extraction method was investigated as described in Chapter 2. The results of absorbances were given in Figure 34. As can be seen from figure, the fermentation of functional sugars in the syrup produced an increase in initial growth rate but no differences were observed in the time of passing to the stationary phase. The respective growth rates were found to be 0.0815 and 0.1025 for standard medium and syrup, respectively. The increase in initial growth rates of microorganism in syrup to standard was found as 1.26.

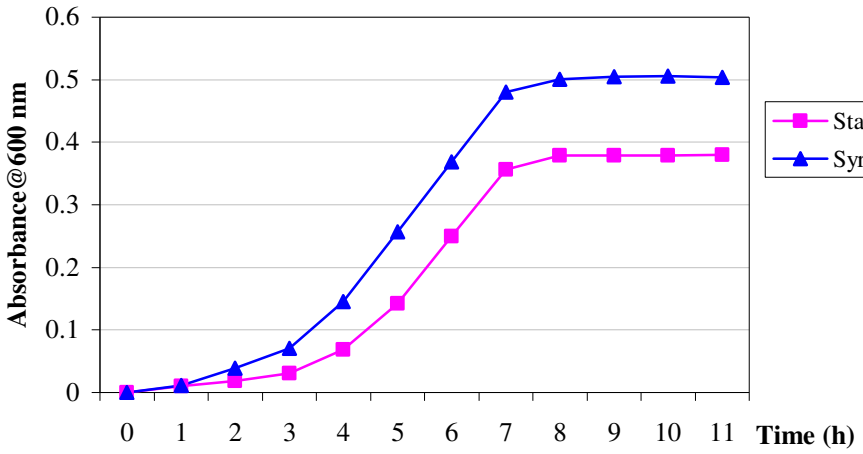


Figure 34. Growth of *Lactobacillus plantarum* 1193 under the standart media containing same amount of waste sugars and the syrup obtained with mid-February-harvested 20-day-stored jerusalem artichokes under acidic conditions

### 3.8 Microwaving

In order to observe the effect of microwaving, same experiments were carried out by using February 2006-harvested, fresh, 1 min-microwaved tubers by following the extraction procedure described in Chapter 2; extractions were carried out at 60°C for 40 min in 40 ml of water.

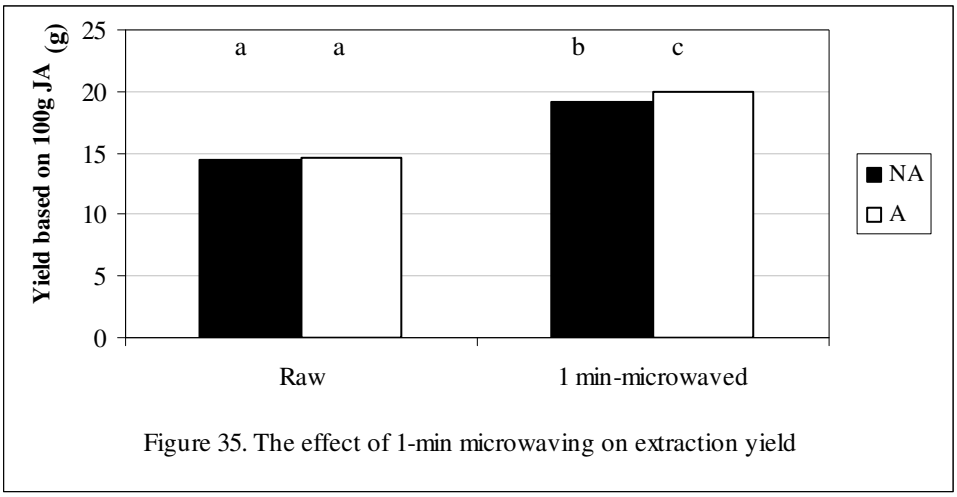


Figure 35. The effect of 1-min microwaving on extraction yield

Higher yield with higher degree of polymerization in both with or without acid added extractions was observed by 1-min microwaving (Figure 35 and 36). This behavior was in contrast with the earlier results of the conventional extractions. The higher degree of polymerization demonstrated that 1-min microwaving caused some decrease in the activity of the enzyme. In fact it was one of the aims of applying microwaving to investigate the contribution of the enzyme to the hydrolysis. The effect of denaturing the enzyme via microwaving has also been stated in the literature [168, 169]. Under acidic conditions the effect of enzyme denaturation was lessened with the hydrolysis effect of the acid added.

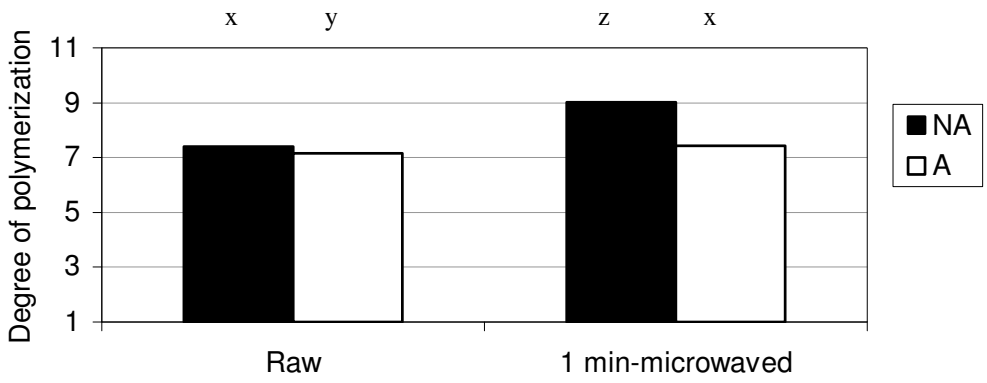


Figure 36. The effect of 1 min-microwaving on degree of polymerization

In HPLC analysis of the syrups obtained by extracting raw and 1 min-microwaved jerusalem artichoke tubers under acidic conditions, the percentages of waste, detected and functional sugars were represented in Figure 37. As can be seen from figure, 1 min-microwaving decreased the percentages of waste and detected sugars. Since it decreased the amount of waste sugars, functionality of the syrup was increased, although the percentage of functional sugars was not changed with microwaving. In fact, the functionality of the syrup was found as 2.65, which was nearly 1.6 times higher than those obtained with raw tubers.

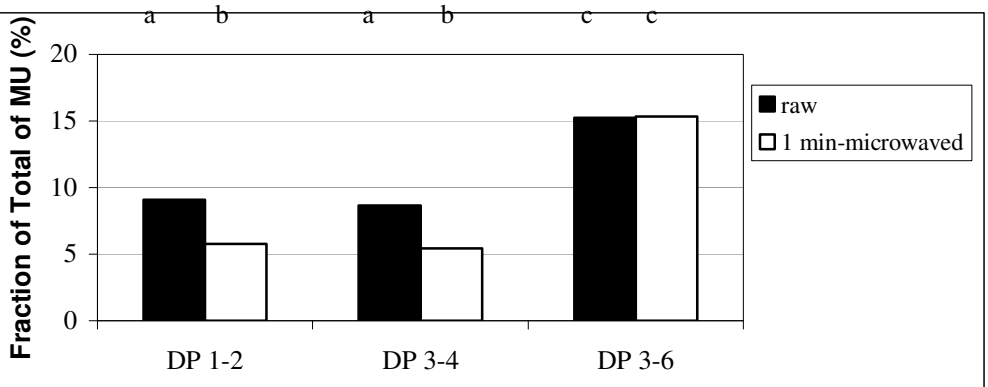


Figure 37. The effect of 1 min-microwaving on distribution of MU in extracts of February 2006-harvested fresh JA tubers under acidic conditions

When the amounts of sugars analyzed by HPLC were considered (Figure 38), it can be seen that microwaving caused glucose production, and a decrease in the amounts of sucrose, 1-kestose and nystose. It also caused some increase in the amounts of sugar with DP of 5, and total monosaccharide units extracted.

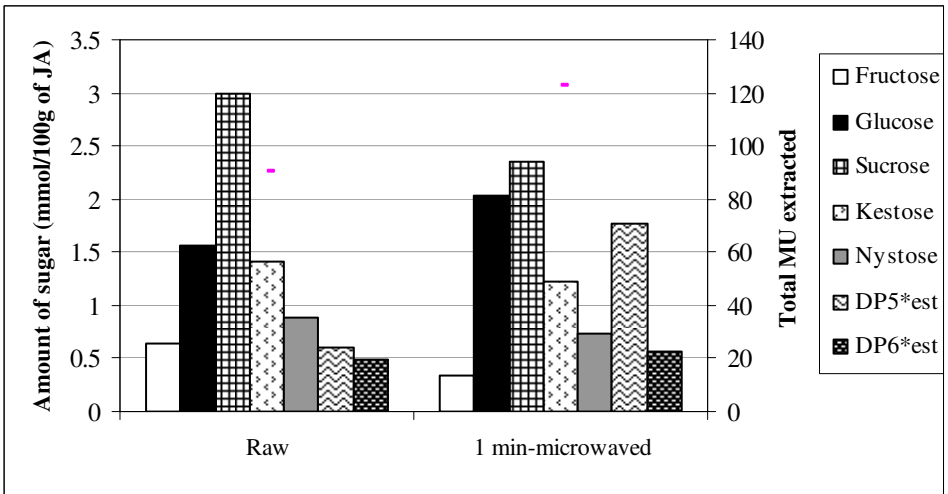


Figure 38. The effect of 1 min-microwaving on product profile of February 2006-harvested fresh JA extracts under A

The density, viscosity, color and darkness of the syrups obtained under acidic conditions were found as 0.98 g/ml, 1.16 cp, 34.16 and 1.2, respectively. By analyzing the data obtained from physical property measurements, it was concluded that 1-min microwaving did not change the density and viscosity of the syrups significantly, but the color and darkness of the syrup were tripled.

The amount of sugars detected or estimated by HPLC that have reducing ends were compared with reducing sugars detected by Nelson-Somogyi method in Figure 39. As it was observed before, the value obtained by HPLC was lower than the one obtained by Nelson-Somogyi Method, since in Nelson-Somogyi method all reducing ends in the extract was determined, while HPLC determines parts of them.

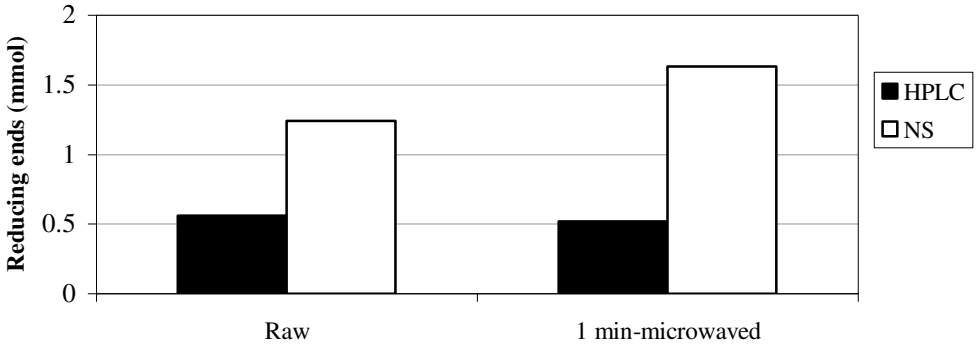


Figure 39. Comparison of amount of sugars as reducing ends obtained from different methods applied to February 2006-harvested raw and 1 min-microwaved fresh JA under acidic conditions

Fermentation of the syrup produced from 1 min-microwaved tubers were also analyzed and the ratio of growth rates was considered. Since the production methods were different for those syrups investigated, the concentrations of waste sugars of the standard media were different. As can be seen from Figure 40, the fermentation of

functional sugars in the syrup produced an increase in growth rate but no differences were observed in the time of passing to the stationary phase, as it was observed before. Thus it was concluded that FOS are equally good substrate as waste sugars, as indicated in the literature [124]. The respective initial growth rates were found to be 0.1375 and 0.1563 for standard medium and syrup, respectively, indicating 1.14 times increase in initial growth rate.

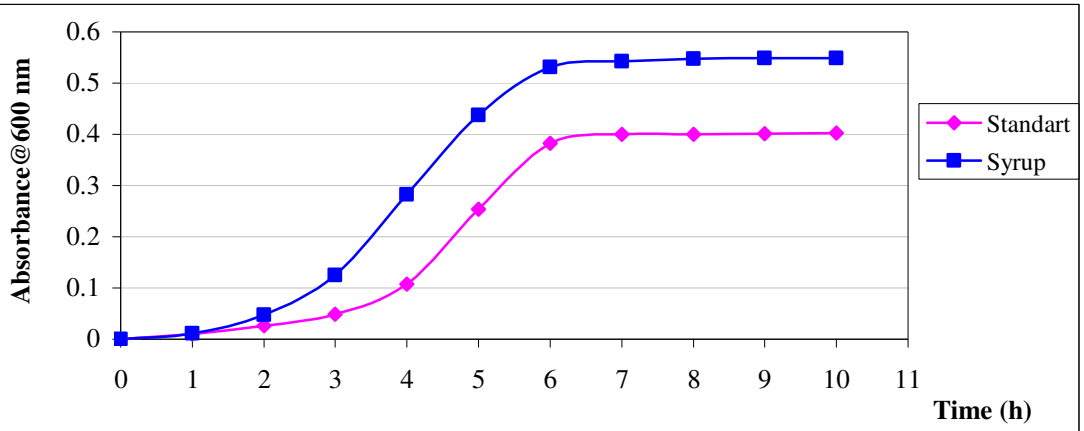


Figure 40. Growth of *Lactobacillus plantarum* 1193 under the standart media containing same amount of waste sugars and the syrup obtained with mid-February-harvested, fresh, 1 min-microwaved jerusalem artichokes under acidic conditions

As a conclusion, it can be said that 1 min-microwaving may be another way of producing these syrups if the increase in color and darkness in the syrup are not problem.

To see the effect of cooking June 2005-harvested 30 day-stored jerusalem artichoke tubers were cooked in microwave oven for 20 minutes, and then the extraction procedure was followed.

Yield values are very low in raw tubers, because of the harvest date and storage time. 1.5 times increase (almost whole dry mater) in the extraction yield was observed by using cooked tubers (Figure 41). This increase can be explained by the cell disruption. In addition, the increasing trend of yield with increasing degree of polymerization in both with or without acid added extractions were observed (Figure 41 and 42).

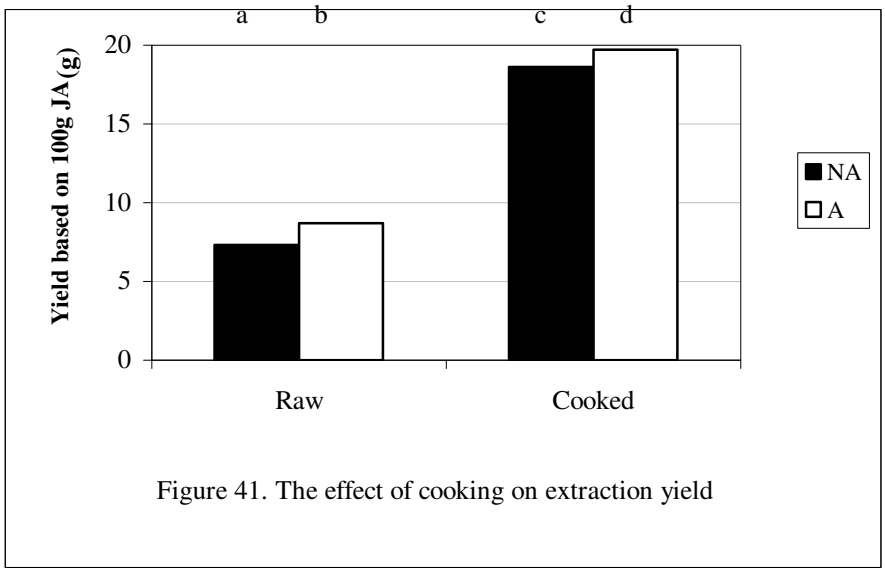


Figure 41. The effect of cooking on extraction yield

The increase in degree of polymerization under non-acidic conditions showed once more that microwaving can achieve enzyme inactivation. The increase in DP observed under acidic conditions is much less, due probably to the contribution of the presence of acid, as was the case with 1 min-microwaving.

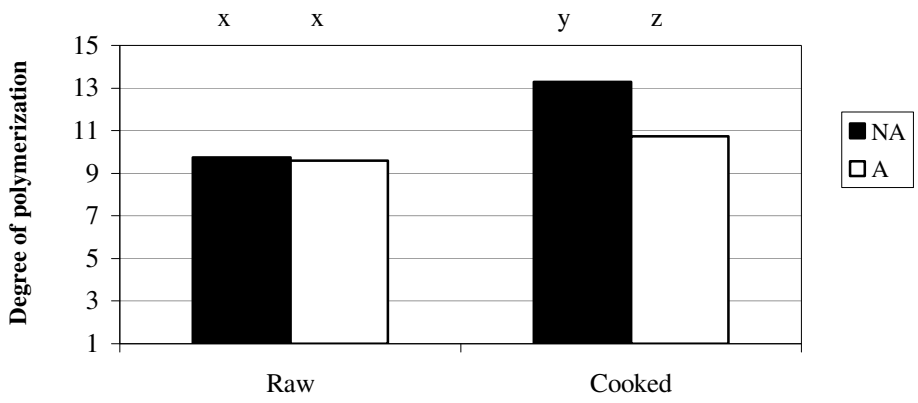


Figure 42. The effect of cooking on degree of polymerization

So, by microwaving of the tubers during 30 min, the syrup yield was increased nearly 1.5 times than microwaving them for 1 min, but the increase in DP was 9 to 13 for NA conditions, and 9 to 11 for acidic conditions compared to 7.4 to 9 for NA, and 7 to 7.4 for acidic conditions of 1 min microwaved tubers. Thus, if the increase in DP up to 13 and the increase in color are not a problem, 30 min microwaving will be desirable much more. But, in cooking cell rupture may be extensive and non-soluble cellulosic material may cause some problems in pumping.

## 3.9 Ultrasonication

In those experiments, 10 g of April 2006-harvested fresh jerusalem artichoke tubers were extracted with 40 ml of water. Ultrasonic extractions were made at 20, 40 and 60°C with the same storage range during 3.5 and 3 minutes under non-acidic and acidic conditions, respectively. The extraction time was reduced because of the

highest yields (2.8g for non-acidic and 4.2g for acidic extractions) and the lowest degree of polymerization (27.3 for non-acidic and 7.2 for acidic extractions) were obtained at these extraction times. In order to make a comparison, water bath extractions were done at same temperature and extraction times.

### 3.9.1 Different Bath Applications & Citric Acid Addition

Since ultrasonic bath was not a temperature controlled, the experiments were done at room temperature first. Then, by heating the solvent at 60°C, the ultrasonic experiments were done in order to compare the results with those obtained by conventional extractions. The results of yield and DP of the syrups produced were represented in Figure 43 and 44 for non-acidic and acidic conditions, respectively.
At 20°C, ultrasonication during extraction resulted in a higher, almost doubled DP under non-acidic conditions (Figure 43), which was probably due to the inactivation of inulinase by ultrasonic waves as it was also stated in the literature [154, 158, 162]. The chromatograms of these syrups were given in Figure E6 in Appendix E. In the case of acidic conditions, however, ultrasonication almost halved the DP, indicating either a stabilizing effect of citric acid on the enzyme or a probable enhancing effect of ultrasonication on acid hydrolysis, because ultrasounds can degrade polysaccharides into parts [157, 159, 160, 161]. The yield was somewhat lowered by ultrasound application in contrast to the literature [159, 160].

At 60°C, 3 min ultrasonication was able to bring about almost 50% of the yield of 40 min non-assisted (conventional) extraction under non-acidic conditions, but in the case of acidic extractions, 3 min ultrasonication achieved nearly 10% increase in the yield of conventional extraction. Two-step extraction, in which the remainder of 40 min after ultrasound application was spent in conventional extraction, caused no improvement neither in yield nor in degree of polymerization. On the contrary, the

extended time increased the degree of polymerization and decreased the functionality under both conditions (Figure 44). Highest functionality of the syrups was obtained under acidic conditions at both 20°C and 60°C.

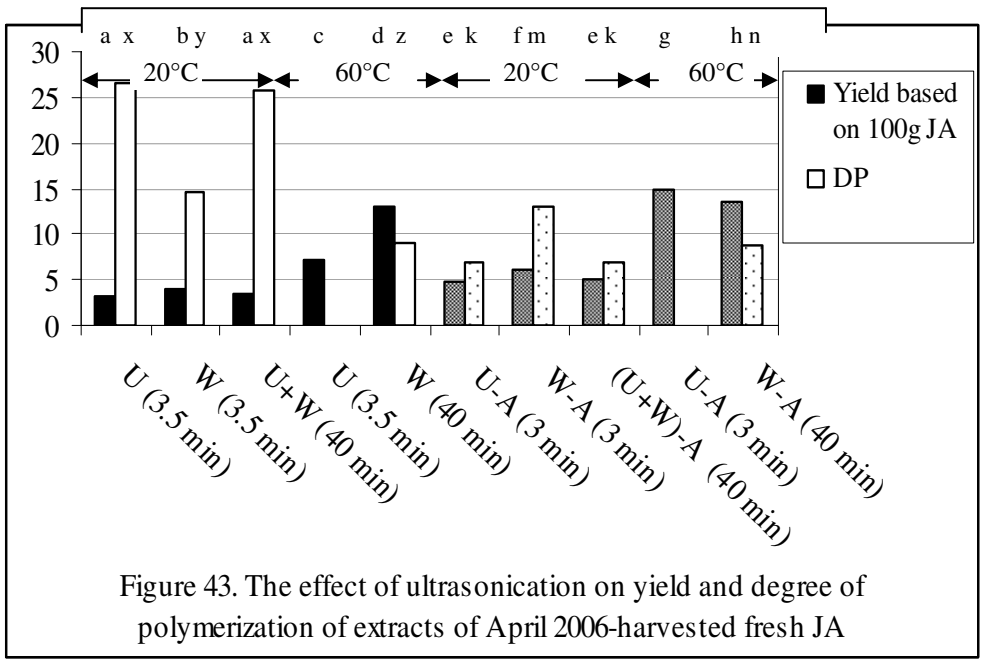


Figure 43. The effect of ultrasonication on yield and degree of polymerization of extracts of April 2006-harvested fresh JA

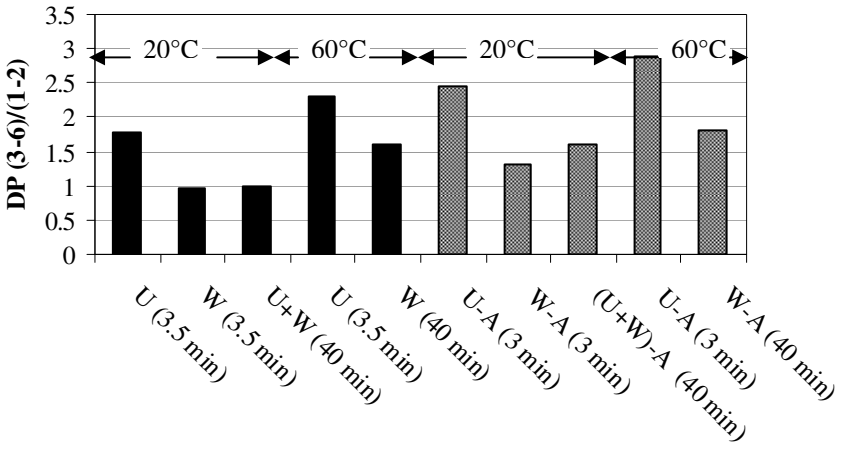


Figure 44. The effect of ultrasonication on functionality of the extracts of April 2006-harvested fresh JA tubers

As can be seen from Table 18, ultrasonication at 20°C and 60°C, increased the ratio of DP (3-4)/(1-2) comparing to conventional and two-step extractions under both non-acidic and acidic conditions. Also, it was found that acid addition increased this ratio in both of the ultrasonic extractions, but the increase was more pronounced at room temperature.

Table 18. The effect of ultrasonication on values of DP (3-4)/ (1-2) of the extracts of April 2006-harvested fresh JA tubers

| Temperature | Bath Application | NA | A |
|---|---|---|---|
| 20°C | Ultrasonic (3.5 min for NA 3 min for A) | 1.45 | 1.96 |
| | Water bath (3.5 min for NA 3 min for A) | 0.91 | 0.65 |
| | Ultrasonic + Water bath (∑ 40 min) | 0.77 | 1.29 |
| 60°C | Ultrasonic (3.5 min for NA 3 min for A) | 1.60 | 1.69 |
| | Water bath (40 min) | 1.35 | 1.57 |

The product profiles obtained under non-acidic and acidic conditions were given in Figure 45 and 46, respectively. Under non-acidic conditions, it can be said that at

room temperature while ultrasonic-bath applications produced some fructose formation, water-bath application produced some glucose formation. In the ultrasonic-bath applied extraction at room temperature no sucrose was observed. At 60°C, ultrasonication caused much more degradation, resulting more waste sugars compared to room temperature, but still lower than those obtained by water bath. The production of functional sugars via ultrasonication was more pronounced with increasing temperature. Application of two-step extraction caused more waste sugars than conventional extraction, and ultrasonication, thus found worse.

Under acidic conditions, similar observations were made for acidic and non-acidic conditions at room temperature, but at 60°C due to the combined effect of acid and ultrasonication on hydrolysis of inulin sucrose formation were observed, while the production of functional sugars was more pronounced. Product profile of syrups obtained by application of two-step extraction was found worse than those of obtained by ultrasonication only. Increasing temperature from 20 to 60°C increased the functional content under both non-acidic and acidic conditions, but higher increase was obtained under acidic extractions. It may due to the additional hydrolysis of acid added or conformational disruption of molecules by acid addition, thereby more degradation via ultrasonic waves.

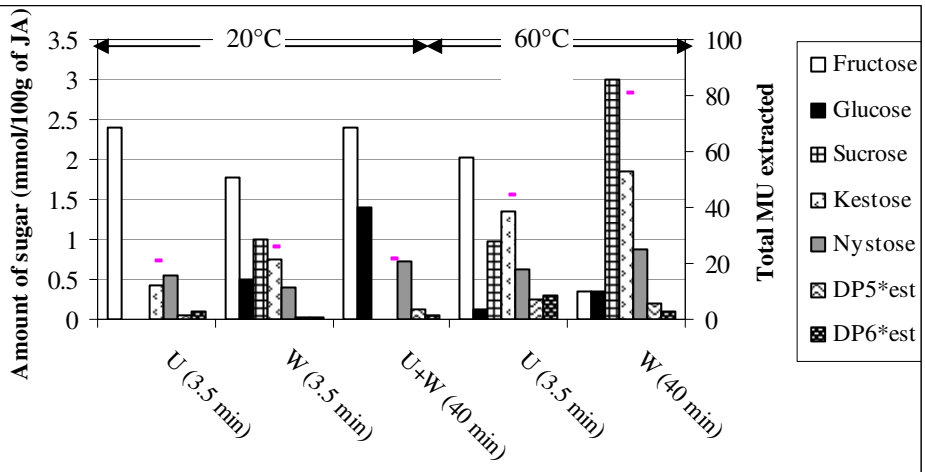


Figure 45. The effect of ultrasonication on product profile of April 2006-harvested fresh JA extracts under NA

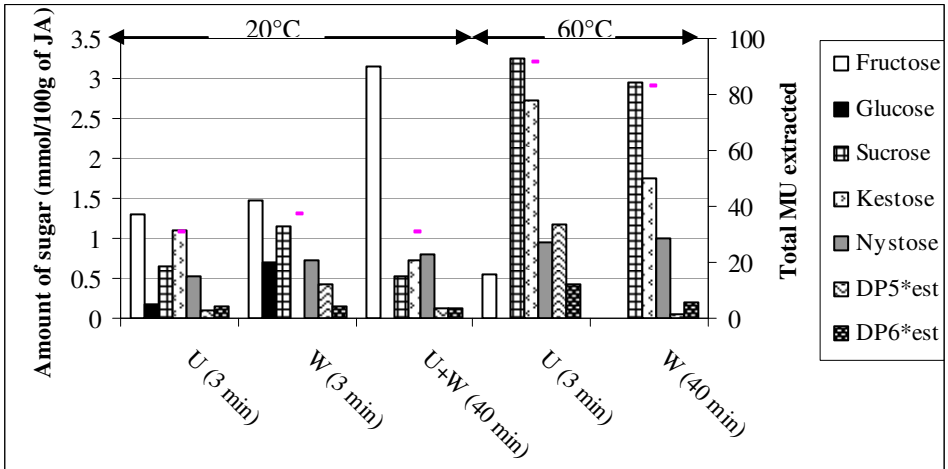


Figure 46. The effect of ultrasonication on product profile of April 2006-harvested fresh JA extracts under A

### 3.9.2 Temperature & Storage Time

To see the effect of storage time and temperature on product profile and functionality of the syrup obtained, the experiments were done with fresh to 20 day-stored April 2006-harvested jerusalem artichoke tubers under non-acidic conditions at 20, 40 and 60°C.

By comparing the product profiles of these extracts obtained under non-acidic conditions, the amount of nystose was observed nearly the same in all temperatures (Figure 47). Production of kestose, and sucrose were observed at 60°C can be explained by product degradation via ultrasonic waves at that temperature, as stated in the literature [157]. Under acidic conditions (Figure 48), the sum of monosaccharide units tripled; the amounts of fructose decreased and glucose disappeared; and the higher sugars increased with increasing the temperature from 20 to 60°C.

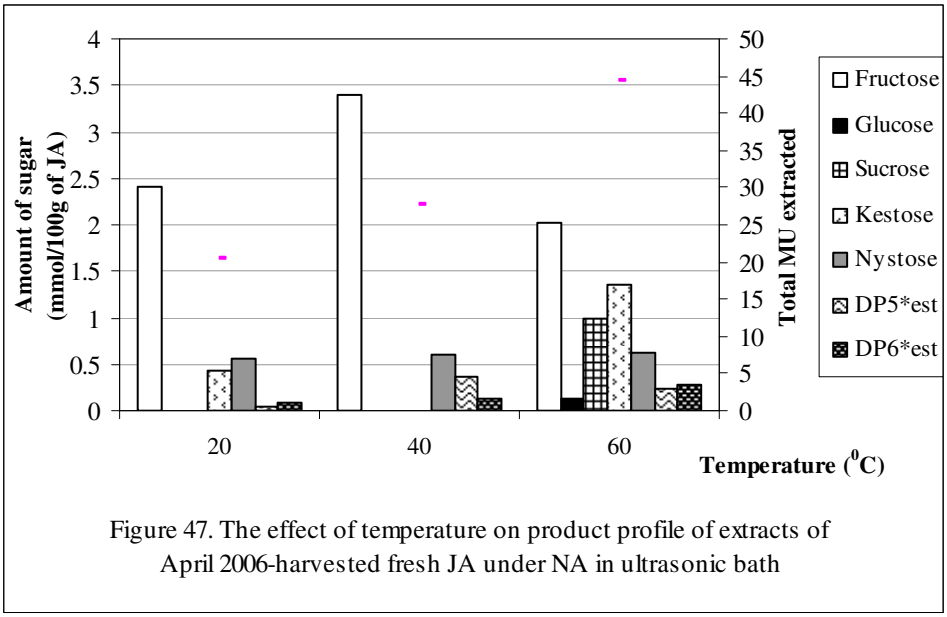


Figure 47. The effect of temperature on product profile of extracts of April 2006-harvested fresh JA under NA in ultrasonic bath

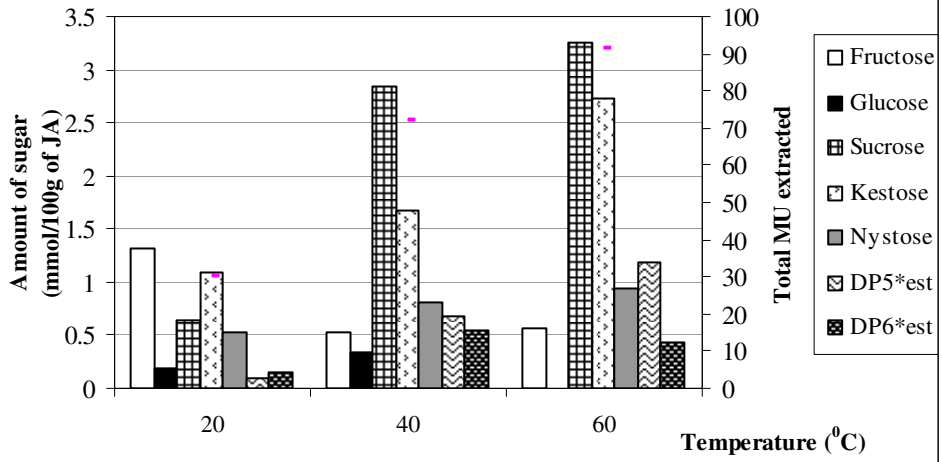


Figure 48. The effect of temperature on product profile of extracts of April 2006-harvested fresh JA extracts under A in ultrasonic bath

The changes in yields of the ultrasonication-assisted extractions with storage time of the tubers were given in Figure 49 and 50 for non-acidic and acidic conditions. As it was observed in conventional water-bath extracts, yield increased with increasing storage time under non-acidic conditions, because of the combined effect of the remaining enzyme activity and ultrasonication itself on degradation of inulin into smaller molecules that can much easily diffuse into the solution. It was observed that, as the temperature increased, yield increased as in the case of conventional extraction, except at 40°C.

Under acidic conditions (Figure 50), as it was observed in conventional water-bath extracts, yield increased with increasing storage time and also temperature, ignoring the extracts of freshly used JA tubers at 40°C. By increasing temperature from 20°C to 60°C, the yield was increased at least 2.5 times. Highest yield (15g) was obtained with fresh JA extracts at 60°C. Comparing the yield obtained with JA tubers having same storage time by ultrasonic extraction under non-acidic conditions (7.2g), it was concluded that acid addition increased the yield of the extract, but same results were not observed in all storage times. The fluctuations observed may resulted from the

combined action of the dynamic fructan metabolism of the tubers and the effects of ultrasonic waves and added citric acid on those molecules.

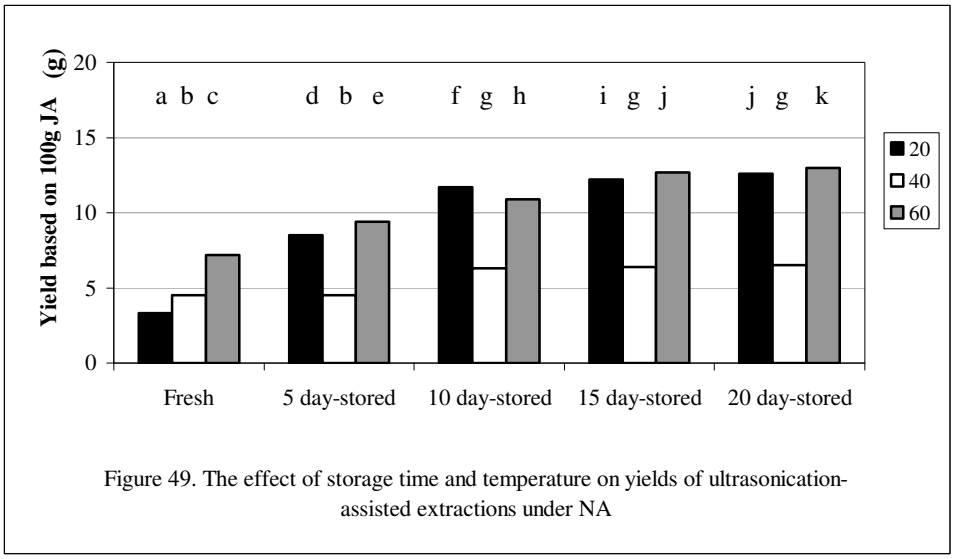


Figure 49. The effect of storage time and temperature on yields of ultrasonication-assisted extractions under NA

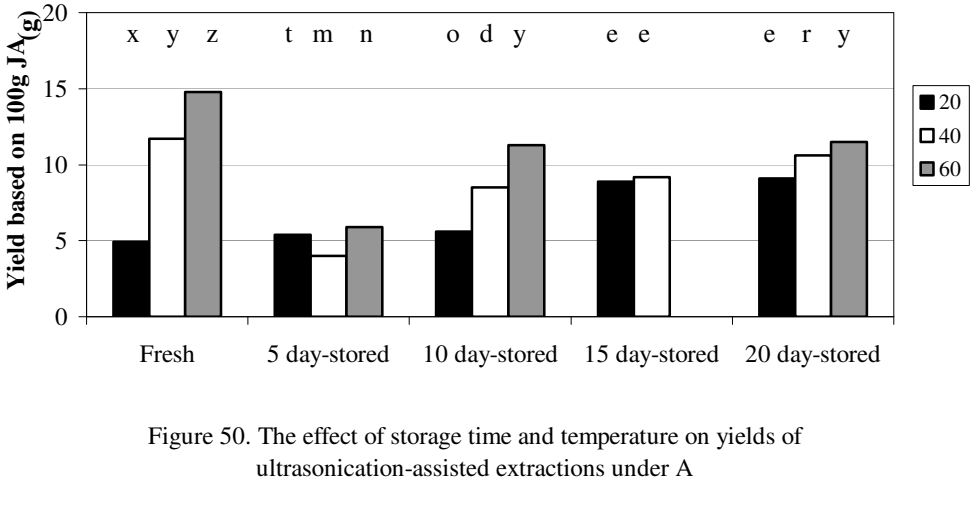


Figure 50. The effect of storage time and temperature on yields of ultrasonication-assisted extractions under A

In Figure 51, the effect of storage time on product profiles of the sugars obtained by ultrasonication-assisted extraction under non-acidic conditions at 60°C was given. Since no waste sugars were produced, the most functional syrups can be produced by extracting 10 day-stored tubers at this temperature. The fluctuations observed may resulted from the combined action of the dynamic fructan metabolism of the tubers and the effect of ultrasonic waves on the compounds that produced due to this fructan hydrolysis. It was also observed that storing up to 20 day, doubled the total MU extracted.

The effect of refrigerated storage on the extracts obtained under acidic conditions (Figure 52) was the same as in non-acidic case; 10 day-stored tubers produced syrups with no glucose, fructose, and sucrose. The largest amounts of total monosaccharide units were obtained 10 day-stored JA under acidic conditions. Comparing the most functional sugars obtained under both non-acidic and acidic conditions (10 day-stored tubers), it was concluded that acid addition increased the functionality of the syrups produced. Additionally, non-calorie sweeteners can be produced with 10-day stored tubers.

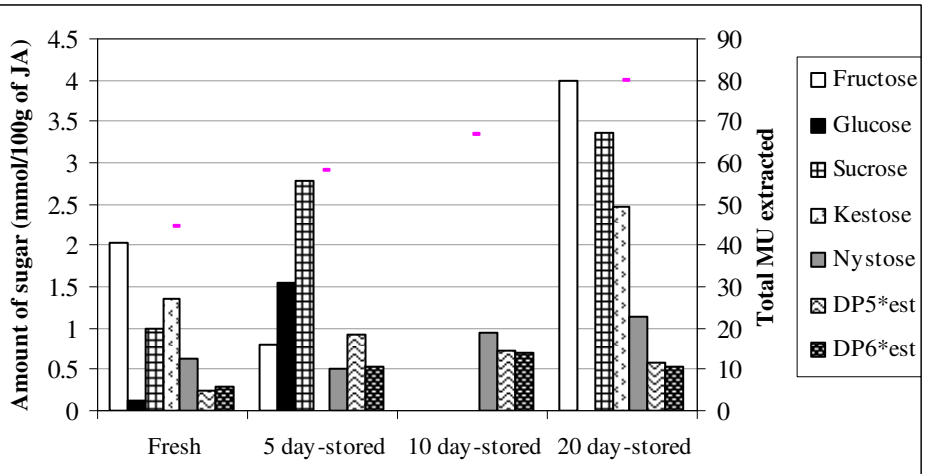


Figure 51. The effect of storage time on product profile of extracts of April 2006-harvested JA extracts under NA at $60^0$C.

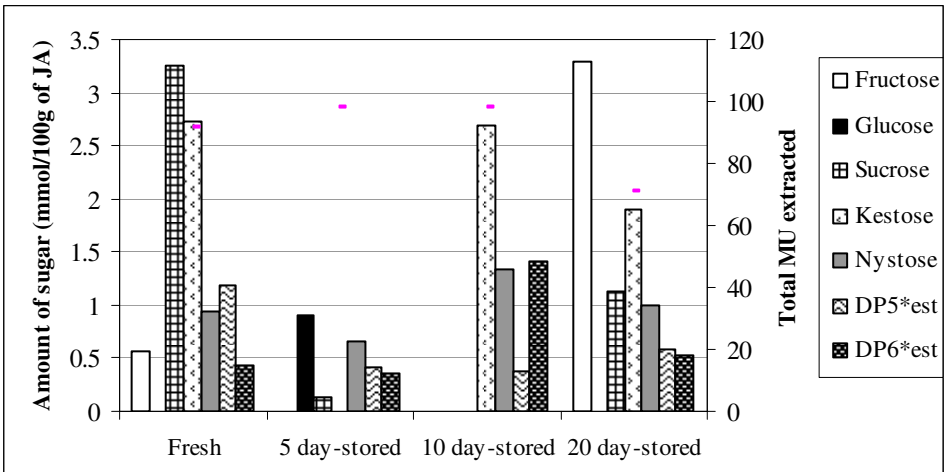


Figure 52. The effect of storage time on product profile of extracts of April 2006-harvested fresh JA extracts under A at $60^0$C

Due to the statistical analysis, storage time and temperature were found ineffective on any of the physical property measured; thus average values were considered. The density, viscosity, darkness and color of the syrups obtained under non-acidic conditions of ultrasonic-bath extractions only at 60°C were found as 0.98±0.02 g/ml, 1.05±0.30 cp, 1.62±0.03, and 38.3±2.10, respectively. If these values were compared with the average values obtained by water-bath experiments under non-acidic conditions (0.97 g/ml, 1.03 cp, 1.89, and 42.3, respectively), it can be said that ultrasonication did not change the physical properties of the syrups produced under non-acidic conditions, except darkness.

Under acidic conditions, the syrup properties were found as 0.99±0.03 g/ml, 1.07±0.17 cp, 0.63±0.26, and 13.68±1.09, respectively. Comparing these values with those obtained under non-acidic ultrasonic extractions at 60°C, it was observed that acid addition did not change density and viscosity significantly but, decreased color (64%) and darkness (61%) of the syrup. If these values were compared with the average values obtained by conventional extractions under acidic conditions (0.98 g/ml, 1.1 cp, 0.38, and 13.3, respectively), it can be said that ultrasonication did not

change the physical properties of the syrups produced, except darkness that was doubled.

So, it was found that ultrasonication increased the functionality of the syrup and depending on the average DP changing with the storage time, it may increase the yield. It did not affect the density, viscosity, and color of the syrups, while doubled the darkness. Citric acid was found to decrease the color of the syrups and also to improve functionality.

It was observed that the most functional syrups via ultrasonication-assisted extraction both under non-acidic (Figure 53) and acidic conditions (Figure 54) can be obtained by extracting 10 day-stored JA tubers at 60°C. The calculated percentages of waste, detected and functional sugars in the syrup were 0%, 5.58%, and 17.27% for non-acidic conditions, and 0%, 10.9%, and 13.68% for acidic conditions, respectively. The functionalities of these syrups were calculated by dividing % of DP 3-6 to 0.01 not zero (in order to make a comparison). The fluctuations obtained in functionality with storage time and temperature can be explained by changing average degree of polymerization of the tubers during dormancy and storage, and due to those different effects of ultrasounds on those molecules. By comparing the values obtained under non-acidic and acidic conditions, it was concluded that acid addition increased the functionality of the syrup.

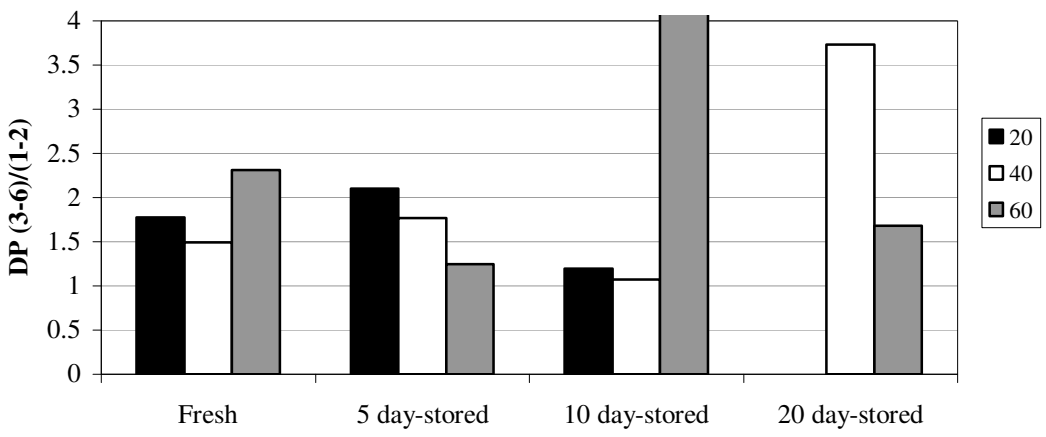


Figure 53. The effect of storage time and temperature on functionality of ultrasonication-assisted extractions under NA

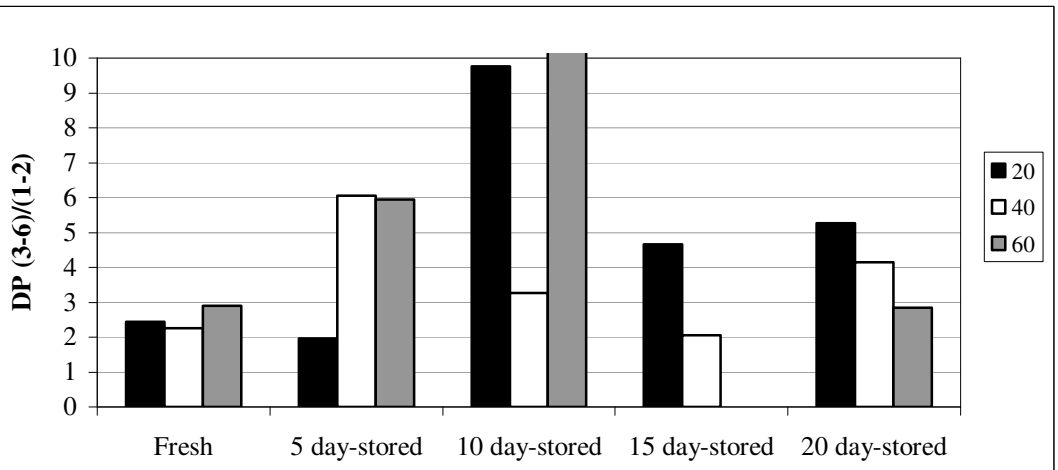


Figure 54. The effect of storage time and temperrature on functionality of ultrasonication-assisted extractions under A

As can be seen from Table 19, the most functional syrup obtained under non-acidic and acidic conditions had the highest ratio of DP (3-4)/(1-2), since no waste sugars were produced.

Table 19. The effect of storage time and temperature on values of DP (3-4)/ (1-2) of the extracts of April 2006-harvested fresh JA tubers

| | 20°C -NA | 20°C-A | 40°C -NA | 40°C-A | 60°C-NA | 60°C-A |
|---|---|---|---|---|---|---|
| Fresh | 1.45 | 1.96 | 0.72 | 1.25 | 1.60 | 1.69 |
| 5 day-stored | 1.21 | 0.70 | 0.86 | 2.08 | 0.25 | 2.30 |
| 10 day-stored | 1.54 | 3.66 | 0.54 | 2.11 | 558.00 | 1930.00 |
| 15 day-stored | NM | 3.14 | NM | 1.13 | NM | NM |
| 20 day-stored | NM | 3.15 | 3.15 | 2.76 | 1.11 | 1.75 |

As indicated before, the most functional syrups via ultrasonication-assisted extraction under acidic conditions can be obtained by extracting 10 day-stored JA tubers at 60°C. Comparing the functionalities of the syrups obtained under optimum conditions of ultrasonic and water-bath extracts, it was concluded that much more functional and non-calorie syrups can be produced via ultrasonication.

Fermentation of syrups obtained by ultrasonic extraction of 20 day-stored jerusalem artichoke tubers with 40 ml water containing 26mM citric acid at room temperature for 3 min were analyzed. The results of absorbances were given in Figure 55.

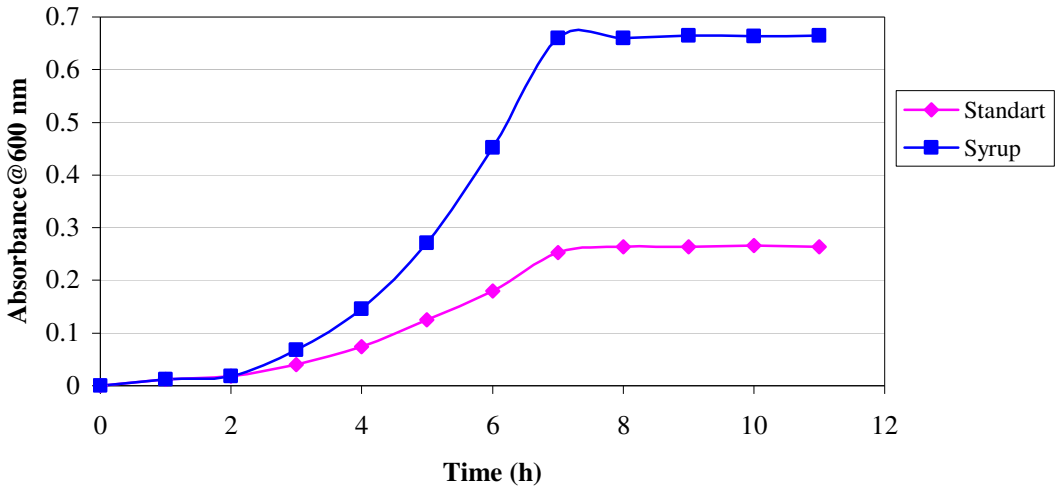


Figure 55. Growth of *L. plantarum* 1193 under the standart media containing same amount of waste sugars and the syrup obtained with April-harvested, 20 day-stored JA extracted with ultrasonic-bath at room temperature under acidic conditions

The growth rate of microorganisms were found to be 0.0597 and 0.171 for standard and syrup, respectively; thus 2.86 times increase in initial growth rates was observed.

As a result of ultrasound applied experiments, optimum conditions were found as 60°C, 3 min, 40ml water containing 26mM acid, April harvested, 10-day stored whole tubers. Under this condition nearly 82% analytical yield was obtained with a solvent to solid ratio of 4. Comparing the results obtained with those of Lingyun and coworkers [166], nearly same yield was obtained by reducing the extraction time and solid to solvent ratio. On the contrary, peeled tubers were used in that study and product profiles did not analyzed.

### 3.10. Comparison of most functional syrups obtained by different methods

By considering all of the experiments under non-acidic and acidic conditions, the most functional syrups were mid-February harvested, 20 day-stored JA extracts for conventional extraction method (at 60°C for 40 min), mid-February harvested, fresh, 1 min-microwaved JA extracts for microwaving, and April-harvested, 10 day-stored JA extracts (60°C for 3 min) for ultrasonic extraction.

The yield, DP, and functionality of the best syrups obtained under different conditions were compared in Figures 56 to 58, respectively. As can be seen from those figures, although ultrasonication yield was lowest under both non-acidic and acidic conditions, it produced much more functional syrups than all the others under non-acidic conditions. It was concluded that the effect of ultrasound on sugar content in the tubers were much more pronounced. Microwaving produced the highest yield and degree of polymerization. The syrup functionality was also increased via microwaving compared to conventional extraction. Thus, it was concluded ultrasonication produced the most functional sugars (as can also be seen in Table 20), with some decrease in yield.

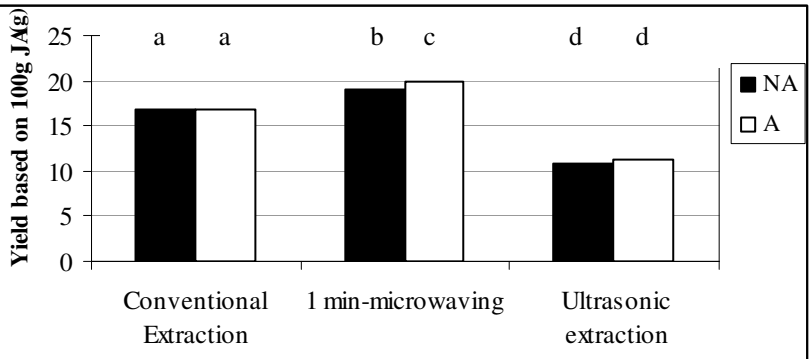


Figure 56. Comparison of yields of the most functional syrups obtained by different applications

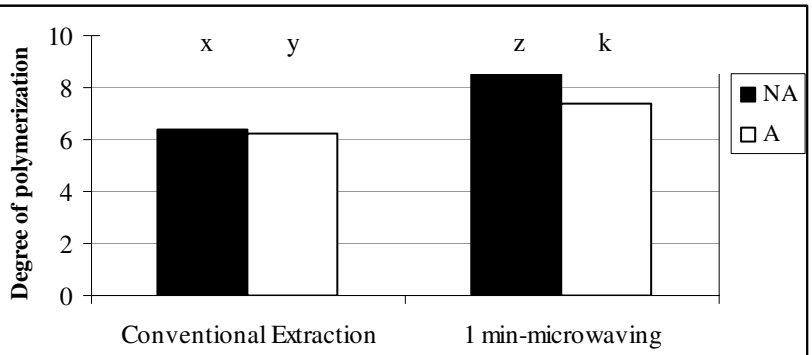


Figure 57. Comparison of degree of polymerization of the most functional syrups obtained by different applications

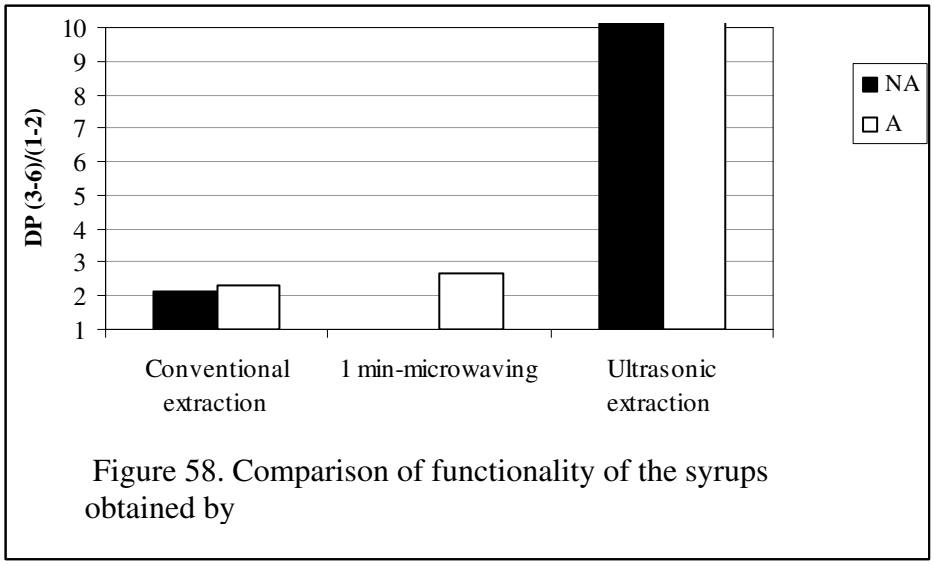


Figure 58. Comparison of functionality of the syrups obtained by

Table 20. Comparison of the values of DP (3-4)/ (1-2) of the most functional syrups obtained by different applications

| | NA | A |
|---|---|---|
| Conventional extraction | 1.36 | 1.44 |
| 1 min- microwaving | NM | 0.94 |
| Ultrasonication | 558.00 | 1930.00 |

By comparing the product profiles, microwaving decreased the amounts of sucrose, while ultrasonication caused no sucrose formation. Applying microwaving the sugars with of DP of 5 were produced, while application of ultrasonication sugars with a DP of 6 were produced. Microwaving decreased the amounts of waste sugars but not disappeared as ultrasonication.

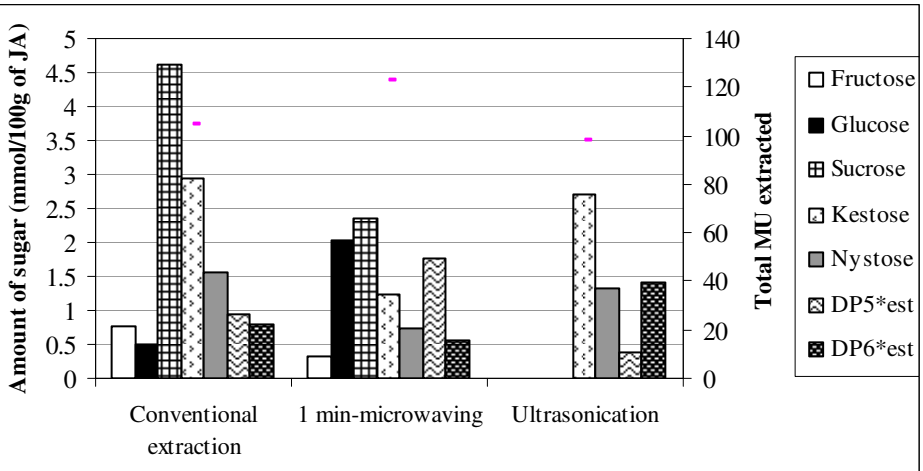


Figure 59. Comparison of product profile of the most functional syrups produced under acidic conditions

The density and viscosity values are practically the same for all syrups (Table 21). Compared to non-assisted extraction, the darkness values are doubled by ultrasonication and tripled by microwaving under acidic conditions. The color was not affected significantly by ultrasonication, but almost tripled by microwaving.

Table 21. Comparison of the physical properties of the most functional syrups obtained by different applications

| | | Conventional extraction | 1 min-microwaving | Ultrasonication |
|---|---|---|---|---|
| NA | Density (g/ml) | 0.97±0.11 | NM | 0.98±0.02 |
| | Viscosity (cp) | 1.03±0.09 | NM | 1.05±0.30 |
| | Color | 42.3±2.5 | NM | 38.30±2.10 |
| | Darkness | 1.89±0.4 | NM | 1.62±0.03 |
| A | Density (g/ml) | 0.98±0.10 | 0.98 | 0.99±0.03 |
| | Viscosity (cp) | 1.10±0.07 | 1.16 | 1.07±0.17 |
| | Color | 13.30±0.70 | 34.20 | 13.70±1.09 |
| | Darkness | 0.38±0.02 | 1.20 | 0.63±0.26 |

Fermentation of functional sugars in the syrup produced an increase in growth rates (Figure 60), but no differences were observed in the time of passing to the stationary phase. Thus it can be said that the functional sugars in syrups produced were as good substrate as simple sugars for the microorganisms chosen. The ratio of respective growth rates for the syrup and standard medium and was found to be 1.26 for 40 min water-bath extraction at 60°C (February 2006-harvested 20 day-stored tubers) 1.14 for tubers microwaved for 1-min prior to water-bath extraction (February 2006-harvested, fresh) and 2.86 for 3 min ultrasonic extraction at 20°C (April 2006-harvested 20 day-stored tubers). The prebiotic contents were verified the most functionality obtained by ultrasonication than microwaving than conventional extraction.

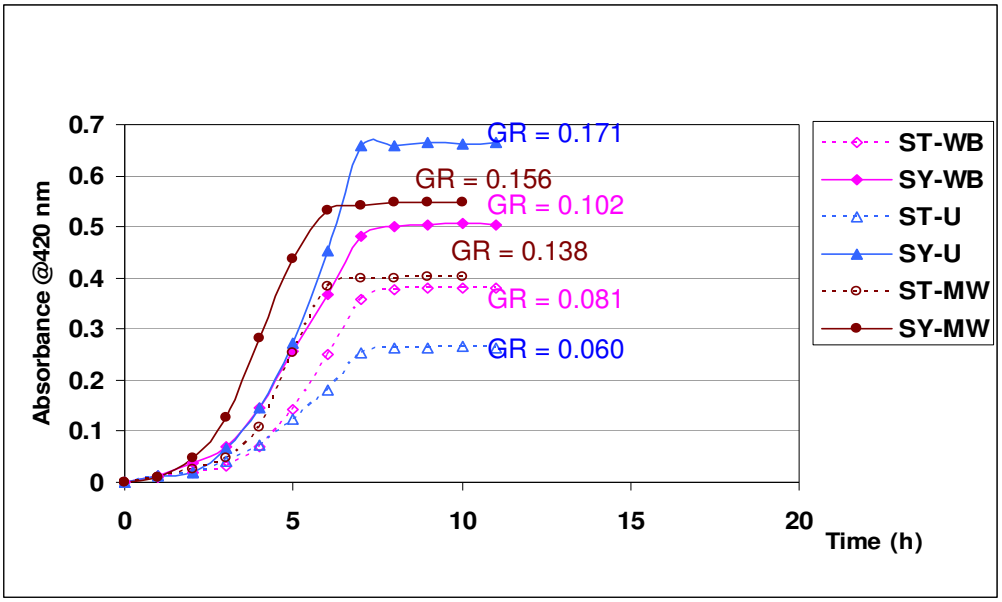


Figure 60. Comparison of the prebiotic property of the syrups obtained by different applications

### 3.11. Consideration of industrial applications

Current sugar production conditions consists of counter-current extraction of 1.2kg of water / kg of grated beets at 70°C during 1h, as it was stated in Chapter 1, whereas the conditions for the production of FOS syrups from 100 were found as 60°C during 40 min with 4 kg of water containing 26mM citric acid / kg of grated jerusalem artichoke tubers. Lowered required extraction time and temperature may reduce the cost of the process. Because of acid addition, color reduction or whitening may not be needed, so the production time, and thus the cost of the whole process may also be decreased. Higher dry matter content of the FOS syrups (nearly 17%) than syrups produced from sugar beets (nearly 14%) is another advantage of the process. Higher solvent to solid ratio of FOS production may not be problem if the product will be used as syrup not a powder. Although the cost of the raw material seems to be higher in FOS production, it is because of the lower production rate. The government

subsidy on sugar beet production may be another reason. It can overcome by encouraging the farmer, since this plant can grow in most of the places in our country. Considering the beneficial effects of FOS, their sweetening power, and similarities of the processes, current sugar industry can be easily modified into FOS production.

If the cost of the JA is paid for the weight of the tubers as in the case of sugar beet, the values of $Y_{JA}$ will be more important, whereas if the cost is paid for the DM content of the tubers the value of $Y_{DM}$ will be more important, since the extracted DM from the same weight of the tubers could be much more because moisture content will decrease with storing in a refrigerator after harvest. Considering all the $Y_{DM}$ data obtained by different extraction methods applied under optimum conditions (Figure 61), DM content of JA tubers were found as 20% generally, but different yields can be obtained due to the effects of ultrasound on different average degree of polymerization in the tubers because of soil and climatic conditions.

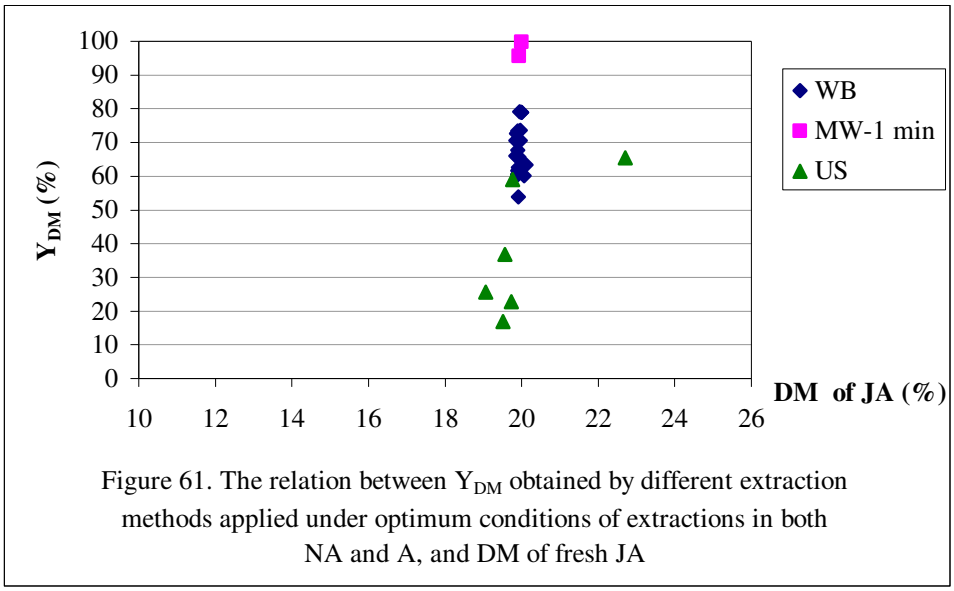


Figure 61. The relation between $Y_{DM}$ obtained by different extraction methods applied under optimum conditions of extractions in both NA and A, and DM of fresh JA

In addition, storing in a refrigerator increased the $Y_{DM}$ values obtained with conventional and ultrasound-assisted extractions under both non-acidic and acidic conditions (Figure 62 and 63), although the DM contents of JA were found as constant (Figure 64 and 65) (except ultrasound-assisted extractions) indicating the occurrence of shorter DP-compounds produced by inulin degradation via inulinase enzyme found in the tubers to met the energy requirement during storage and also because of the effect ultrasound.

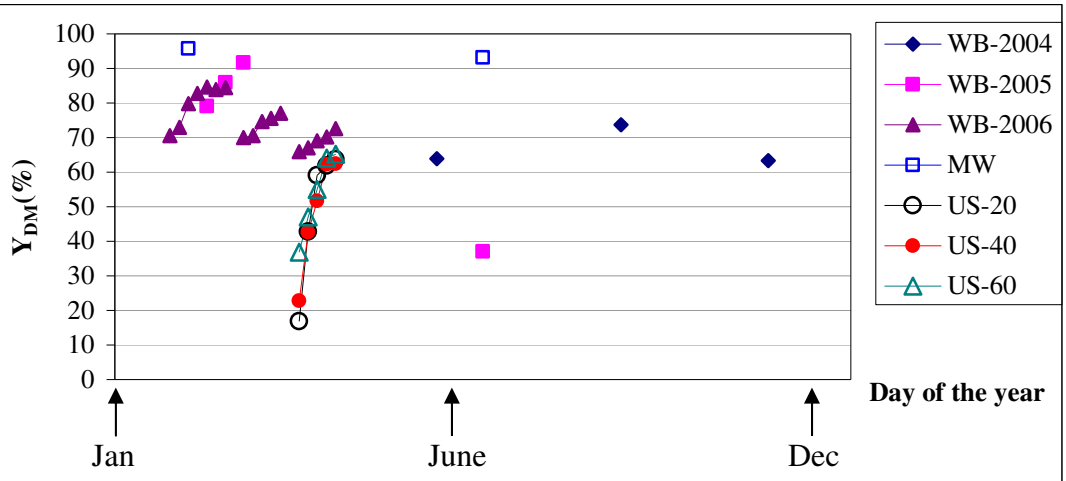


Figure 62. The change of $Y_{DM}$ obtained by different extraction methods under NA with harvest date and storage time

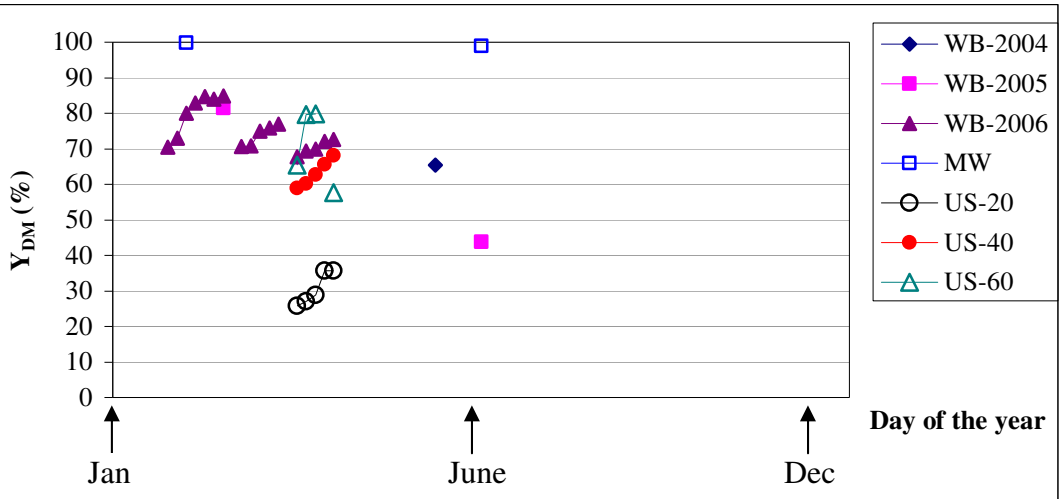


Figure 63. The change of $Y_{DM}$ obtained by different extraction methods under A with harvest date and storage time

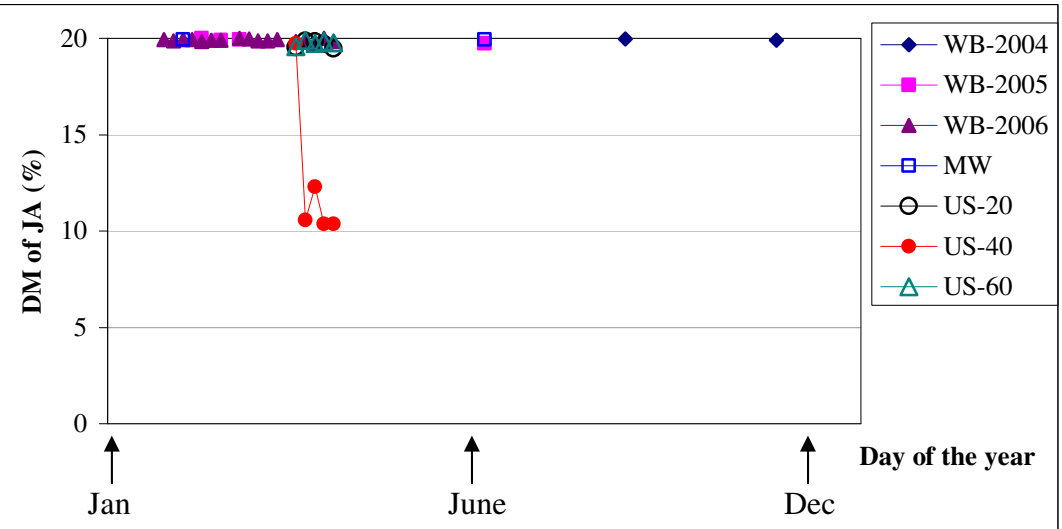


Figure 64. The change of DM content of JA used in different extraction methods under NA with harvest date and storage time

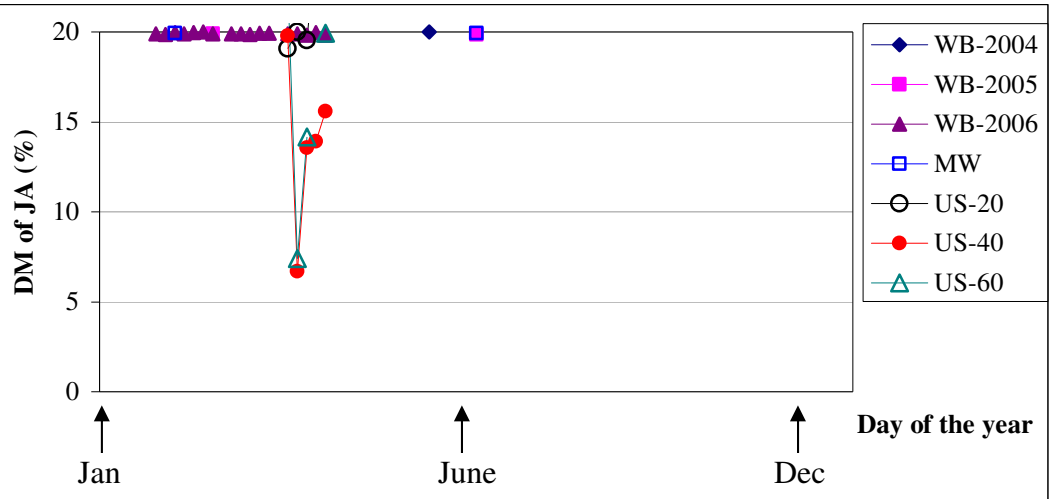


Figure 65. The change of DM content of JA used in different extraction methods under A with harvest date and storage time

The increase in $Y_{JA}$ of the extracts obtained under both non-acidic and acidic conditions was observed with increasing storage time (Figure 66 and 67). The fluctuations observed in the extracts of ultrasound assisted experiments obtained under acidic conditions may result from the combined effects of acid and ultrasound on hydrolysis.

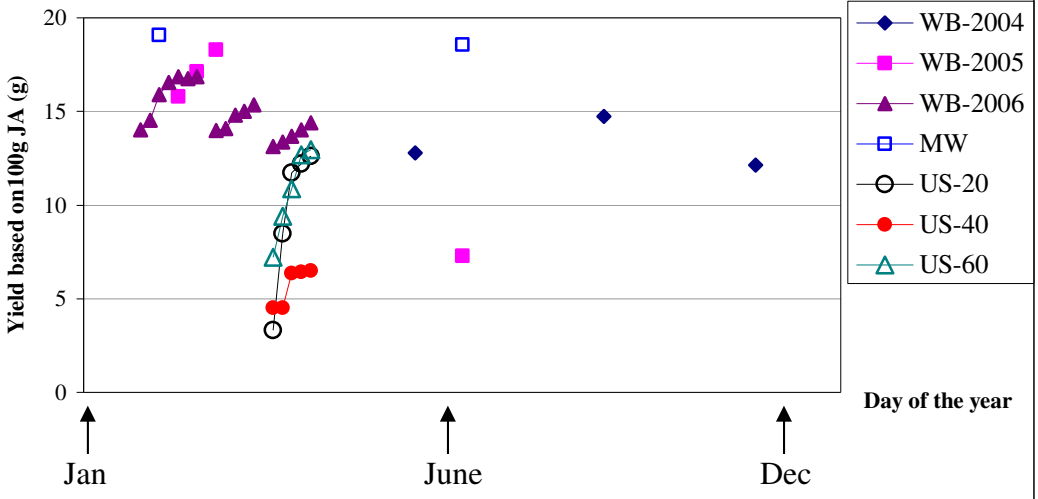


Figure 66. The change of $Y_{JA}$ obtained by different extraction methods under NA with harvest date and storage time

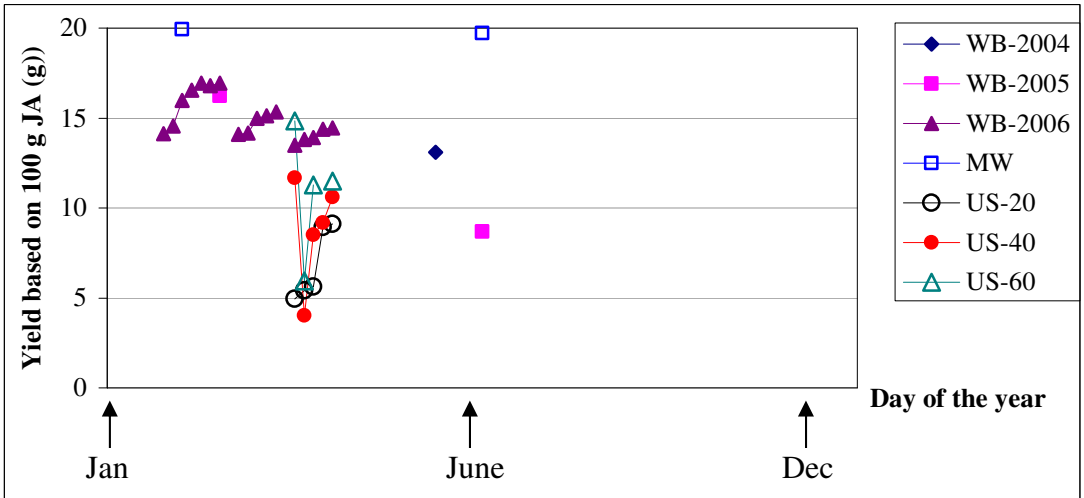


Figure 67. The change of $Y_{JA}$ obtained by different extraction methods under A with harvest date and storage time

# CHAPTER 4

# CONCLUSION

Juicing the tubers produced syrup with DP 33, thus juicing was not a good choice for production of these syrups. In the production of functional syrups from jerusalem artichoke tubers by water extraction at 20-60°C. The optimum conditions for the extractions were found to be 60°C, 40 min, 40 ml of water / 10g of JA containing 26mM citric acid and the use of tubers grated without peeling. Under those conditions, depending on the harvest date, storage time, weather and soil conditions, the syrups having 12g to 17g yield based on 100g of JA tuber, and a DP of 6 to 7 were obtained. The best syrup that has highest yield and functionality, and lowest DP was obtained with the extraction of mid-February 06-harvested 20-day stored samples. Storing in the soil up to mid-February was found a better alternative to storing in the refrigerator under both acidic and non-acidic conditions. Temperature and storage time were found to improve yield and functionality. Citric acid, at 26 mM, improved the color and darkness by 70 and 80%, respectively, while the effects of harvest date and storage time on physical properties of the syrups were insignificant. It was also observed that acid addition did not change the yield, but it may decrease the DP and functionality of the syrup produced depending on the average DP in JA sample changing with weather and soil conditions.

Considering the beneficial effects of FOS, and their sweetening power, and similarities of the processes, current sugar industry can be easily modified into FOS production.

Short-time (1 min) microwaving prior to 40min extraction in shaking water bath, to investigate the contribution of the enzyme to the hydrolysis, increased both the yield

(20%) and degree of polymerization (50%), especially in the non-acidic conditions. The syrup functionality was also increased via microwaving compared to conventional extraction. Application of microwaving produced the sugars with DP of 5, and the amounts of waste sugars were decreased, however the color and darkness of the syrups were tripled by microwaving. Long-time microwaving (20 min) produced about the same increase in yield and in DP.

Ultrasound-assisted-extraction (USE) gave best performance at only 3 min duration; decreased the amounts of sugars with DP 1-2, increased the amounts of functional sugars, although with 18% decrease in the yield. It was found that ultrasonication increased the functionality of the syrup, especially increasing the amounts of sugars with DP 6, it did not affect the density, viscosity, and color of the syrups, while doubled the darkness. Citric acid addition into ultrasonication-assisted extractions was found to decrease the color of the syrups and also to improve functionality. The application of ultrasonication at 60°C compared to 20°C almost tripled the amounts of functional sugars. In order to obtain the largest proportion of monosaccharide units as functional sugars, 10 day storage at 4°C after harvest was indicated.

Fermentation of functional sugars in the syrups verified their prebiotic contents producing an increase in growth rates. The growth rate improvement was highest in USE syrups, followed by syrups obtained by the application of microwaving, and followed by those of conventional extraction. No differences were observed in the time of passing to the stationary phase indicating that the functional sugars in syrups produced were as good substrate as simple sugars for the microorganism chosen.

This was the first study investigating the production of FOS from JA tubers by the action of its native inulinase enzyme and also the effect of microwaving on this production in the literature.

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# APPENDIX A

## FIGURES

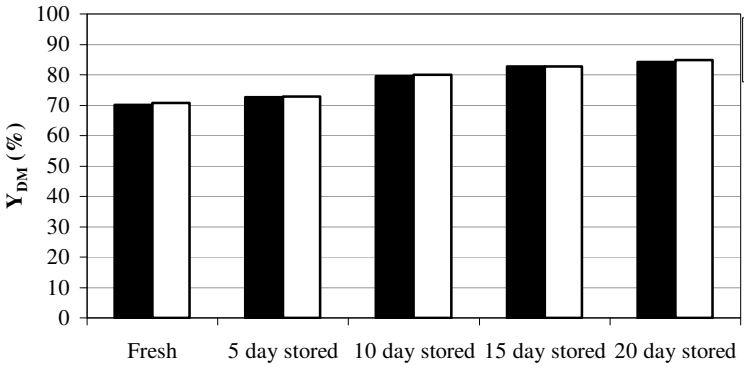



Figure A1.The effect of citric acid addition on yield of extracts obtained by conventional extraction of January 2006-harvested JA tubers

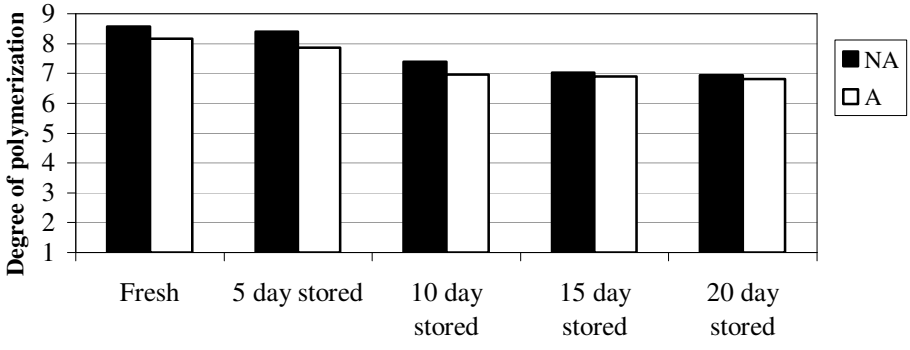



Figure A2. The effect of citric acid addition on degree of polymerization of extracts obtained by conventional extraction of January 2006-harvested JA tubers

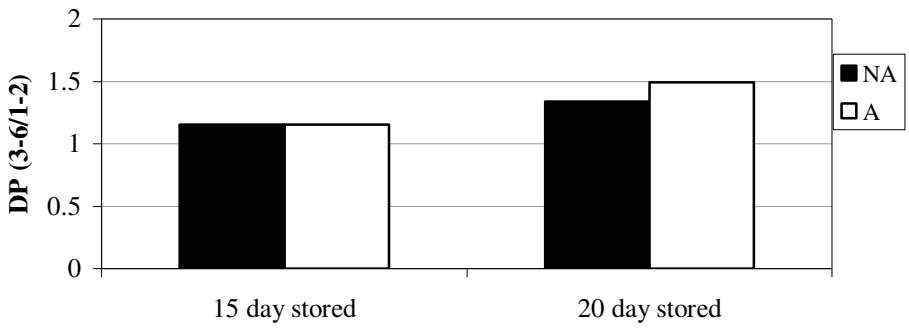
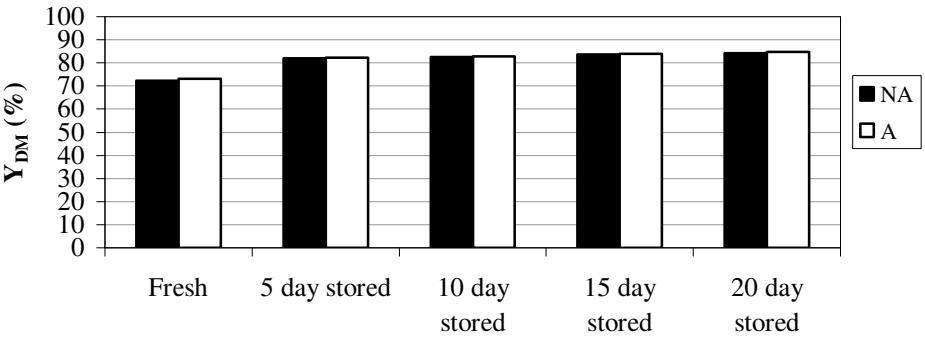



Figure A3. The effect of citric acid addition on functionality of the extracts obtained by conventional extraction of January 2006-harvested JA tubers

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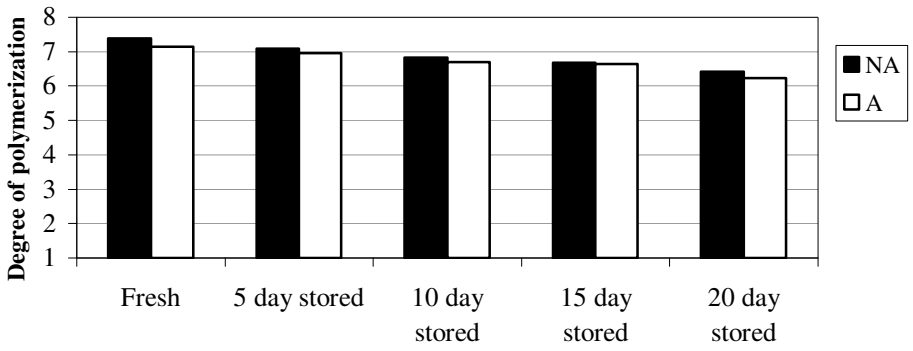


Figure A5. The effect of citric acid addition on degree of polymerization of extracts obtained by conventional extraction of February 2006-harvested JA tubers

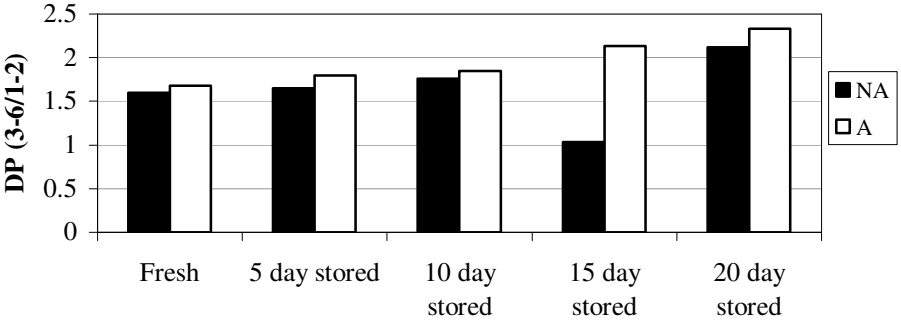


Figure A6. The effect of citric acid addition on functionality of the extracts obtained by conventional extraction of February 2006-harvested JA tubers

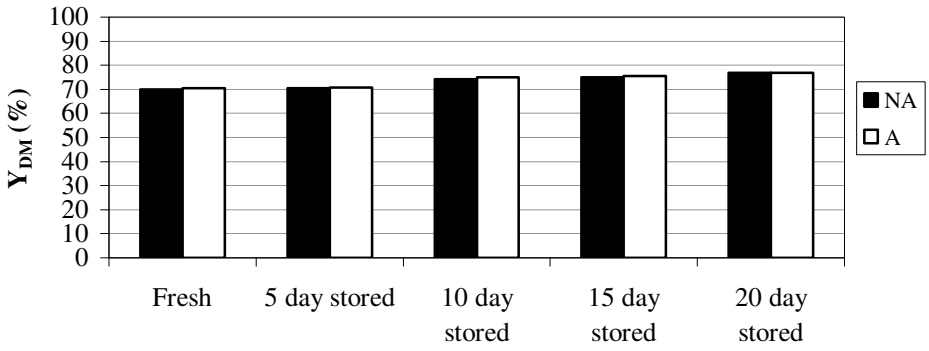


Figure A7.The effect of citric acid addition on yield of extracts obtained by conventional extraction of March 2006-harvested JA tubers

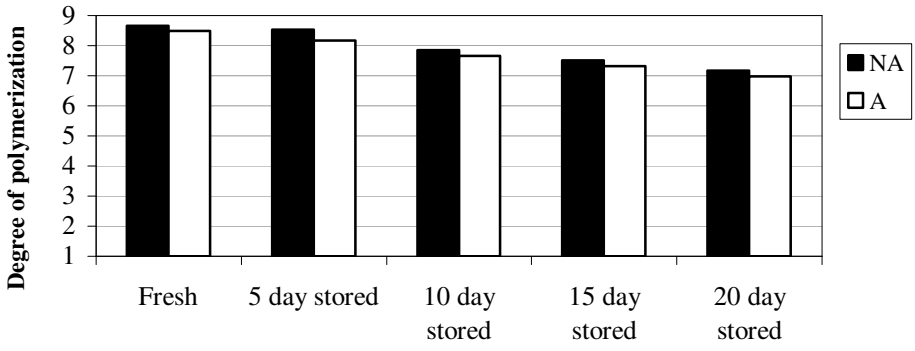


Figure A8. The effect of citric acid addition on degree of polymerization of extracts obtained by conventional extraction of March 2006-harvested JA tubers

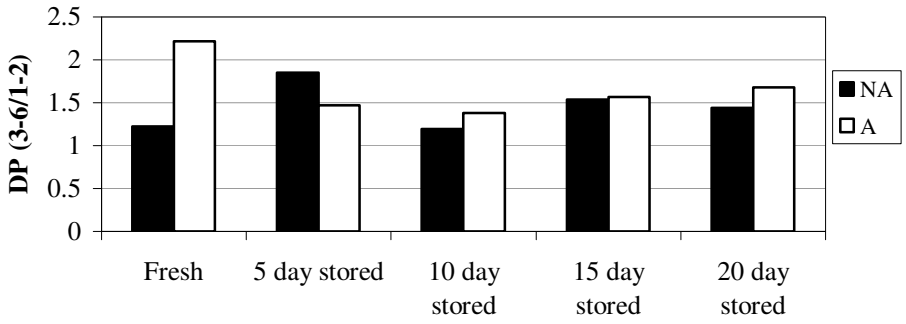


Figure A9. The effect of citric acid addition on functionality of the extracts obtained by conventional extraction of March 2006-harvested JA tubers

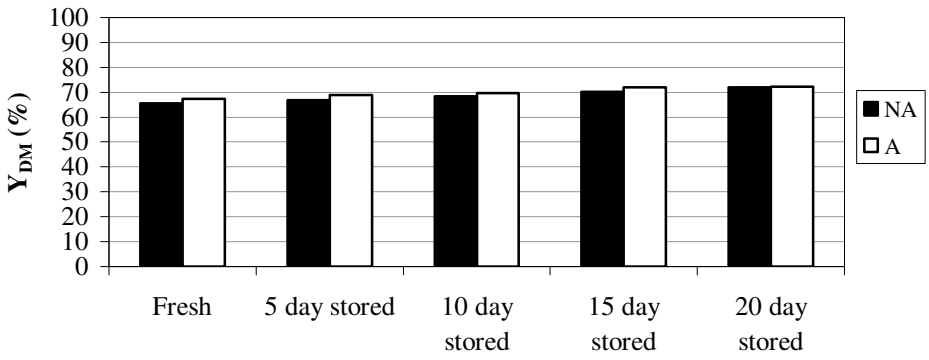


Figure A10. The effect of citric acid addition on yield of extracts obtained by conventional extraction of April 2006-harvested JA tubers

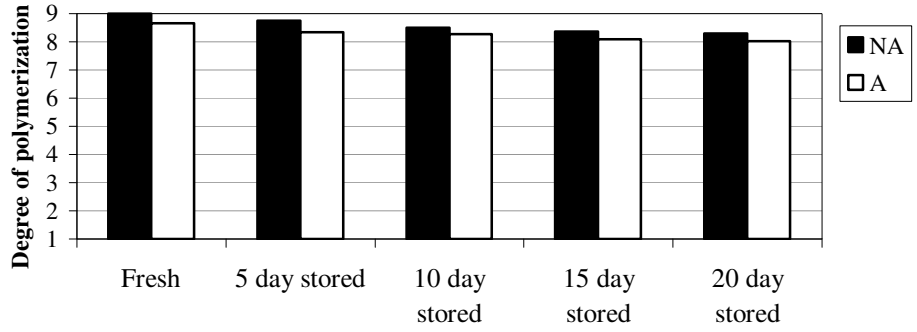


Figure A11. The effect of citric acid addition on degree of polymerization of extracts obtained by conventional extraction of April 2006-harvested JA tubers

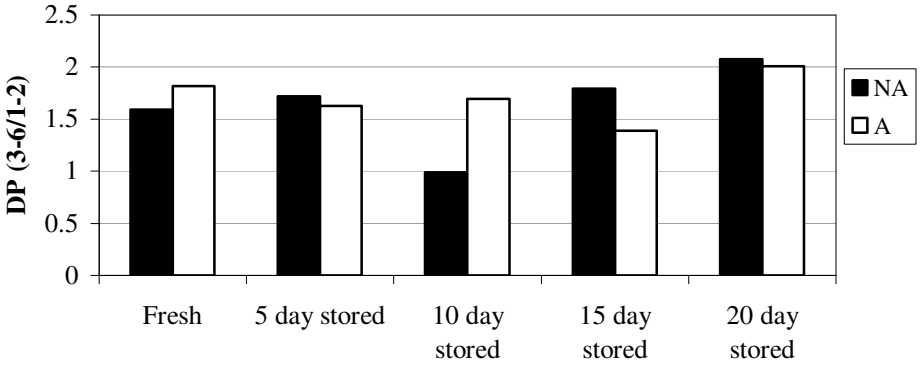


Figure A12. The effect of citric acid addition on functionality of the extracts obtained by conventional extraction of April 2006-harvested JA tubers

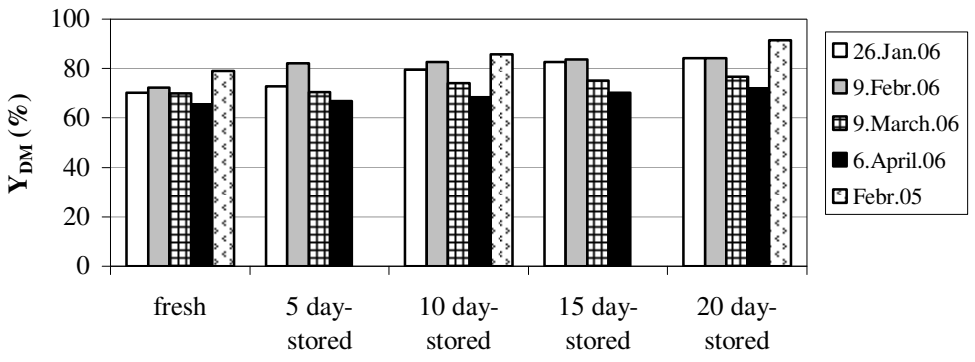


Figure A13. The effect of harvest date and storage time on yield of extracts obtained by conventional extraction in years 2005 & 2006

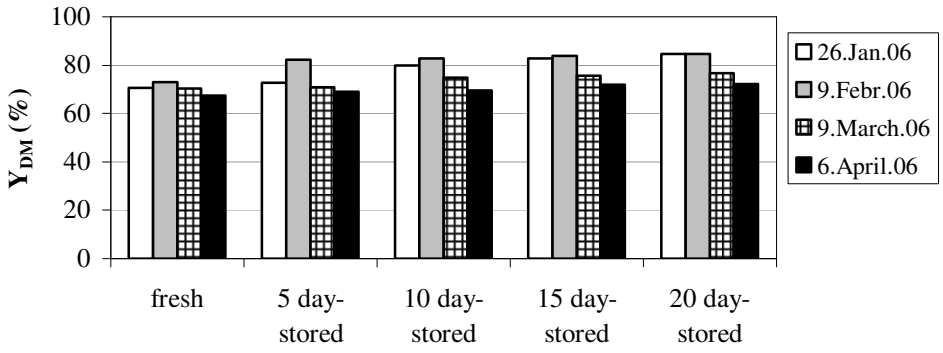


Figure A14. The effect of harvest date and storage time on yield of extracts obtained by conventional extraction under acidic conditions in 2006

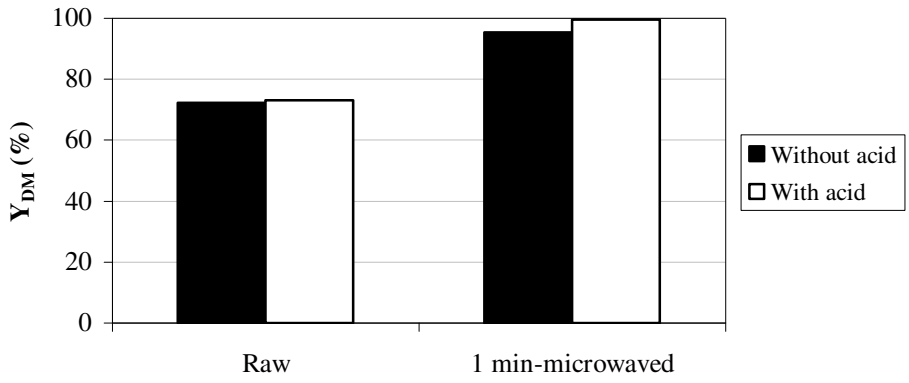


Figure A15. The effect of 1 min-microwaving on extraction yield

# APPENDIX B

## PROCEDURE AND CALCULATIONS OF NELSON-SOMOGYI METHOD

The samples were diluted about 0,002. 2 ml of solution, which prepared by mixing 1 volume of solution B and 4 volumes of solution A, was mixed with the 2 ml of hydrolysis sample. A blank solution was prepared by 2 ml of distilled water. All samples were run three parallels. The blank and solutions were heated at the bath at 100 $^0$C for 20 minutes, then solutions allowed for cooling. After cooling, 1 ml of solution C was added to each of them. Blank solution was used for calibration of the spectrophotometer. Then the absorbance values of samples were recorded at 520 nm.

**SOLUTION A:** 12g Na-K Tartarate, 24g $Na_2CO_3$, 16g $NaHCO_3$, 144g $Na_2SO_4$, Complete this solution to 800 ml.

**SOLUTION B:** 4g $CuSO_4$, 36g $Na_2SO_4$, Complete this solution to 200 ml.

**SOLUTION C:** 5g Ammonium molibdate, 0.6g $Na_2HAsO_4.7H_2O$ was dissolved in 90 ml of Distilled water, then mix 4.2 ml $H_2SO_4$ with 5 ml of distilled water, wait 25 minutes at 55 $^0$C in water bath.

The raw data obtained from the spectrophotometer were in terms of absorbance. The calibration curve of glucose was shown in Figure B1. The obtained absorbance values were translated into concentration (mg glucose) by dividing with the calibration constant (0,143) and multiplying with the volume of sample (40 ml). Reducing end values were obtained.

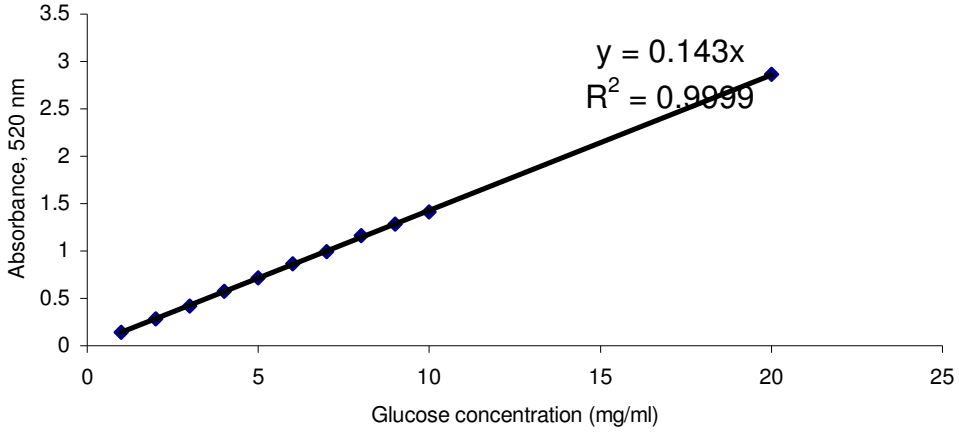


Figure B1. Calibration curve of glucose for Nelson-Somogyi Method

# APPENDIX C

## RAW DATA

### EXPERIMENT NO: 1

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, uncooked, whole

Absorbance@520nm: 0.6509
Yield based on 100g JA: 13.09 g
∑MU extracted: 8.066 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$: 65.47%
DP: 7.88

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

### EXPERIMENT NO: 2

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, NA, uncooked, whole

Absorbance@520nm: 0.7279
Yield based on 100g JA: 12.77 g
∑MU extracted: 7.869 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:63.87%
DP: 6.86

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 3**

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 6.5 mM acidic, uncooked, whole

Absorbance@520nm: 0.5008
Yield based on 100g JA: 10.53 g
∑MU extracted: 6.485 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

$Y_{DM}$: 52.64%
DP: 8.24

Density: ND
Absorbance@420 nm: ND

Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 4**

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 13 mM acidic, uncooked, whole

Absorbance@520nm: 0.5410
Yield based on 100g JA: 10.68 g
∑MU extracted: 6.579 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

$Y_{DM}$:53.40%
DP: 7.73

Density: ND
Absorbance@420 nm: ND

Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 5**

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 19.5 mM acidic, uncooked, whole

Absorbance@520nm: 0.5270

$Y_{DM}$:54.27%

Yield based on 100g JA: 10.85 g DP: 8.07
∑MU extracted: 6.686 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND Viscosity: ND
Absorbance@420 nm: ND ΔE: ND

**EXPERIMENT NO: 6**

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 32.5 mM acidic, uncooked, whole

Absorbance@520nm: 0.6058 $Y_{DM}$: 59.56%
Yield based on 100g JA: 11.91 g DP: 7.70
∑MU extracted: 7.338 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND Viscosity: ND
Absorbance@420 nm: ND ΔE: ND

**EXPERIMENT NO: 7**

Description: June 04 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 39 mM acidic, uncooked, whole

Absorbance@520nm: 0.5799 $Y_{DM}$: 48.99%
Yield based on 100g JA: 9.80 g DP: 6.60
∑MU extracted: 6.035 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND Viscosity: ND
Absorbance@420 nm: ND ΔE: ND

**EXPERIMENT NO: 8**

Description: October 04 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.8398
Yield based on 100g JA: 14.74 g
∑MU extracted: 9.079 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:73.69%
DP: 6.86

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 9**

Description: October 04 harvested, fresh, 40 ml, 40°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.5839
Yield based on 100g JA: 12.04 g
∑MU extracted: 7.417 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:60.20%
DP: 8.08

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 10**

Description: October 04 harvested, fresh, 40 ml, 50°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.7512
Yield based on 100g JA: 14.11 g
$Y_{DM}$:70.55%
DP: 7.35

∑MU extracted: 8.692 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 11**

Description: October 04 harvested, fresh, 40 ml, 70°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.7089
$Y_{DM}$:65.68%
Yield based on 100g JA: 13.14 g
DP: 7.25
∑MU extracted: 8.092 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 12**

Description: October 04 harvested, fresh, 40 ml, 80°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6287
$Y_{DM}$:60.63%
Yield based on 100g JA: 12.13 g
DP: 7.55
∑MU extracted: 7.470 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 13**

Description: December 04 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: ND
Yield based on 100g JA: 12.68 g
∑MU extracted: 7.811 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:63.40%
DP: ND

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 14**

Description: December 04 harvested, fresh, 30 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: ND
Yield based on 100g JA: 12.11 g
∑MU extracted: 7.457 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:60.53%
DP: ND

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 15**

Description: December 04 harvested, fresh, 35 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm:ND
Yield based on 100g JA: 12.34 g
$Y_{DM}$: 61.70%
DP: ND

∑MU extracted: 7.601 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 16**

Description: December 04 harvested, fresh, 45 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$: 62.63%
Yield based on 100g JA: 12.53 g
DP: ND
∑MU extracted: 7.716 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 17**

Description: December 04 harvested, fresh, 50 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$: 53.97%
Yield based on 100g JA: 10.79 g
DP: ND
∑MU extracted: 6.649 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 18**

Description: February 05 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9394
Yield based on 100g JA: 15.80 g
∑MU extracted: 9.733 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$: 79.00%
DP: 6.57

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 19**

Description: February 05 harvested, fresh, 40 ml, 60°C, 10 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.5956
Yield based on 100g JA: 12.11 g
∑MU extracted: 8.455 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$: 60.51%
DP: 7.92

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 20**

Description: February 05 harvested, fresh, 40 ml, 60°C, 20 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6927
Yield based on 100g JA: 13.10 g
$Y_{DM}$: 65.49%
DP: 7.40

∑MU extracted: 8.068 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 21**

Description: February 05 harvested, fresh, 40 ml, 60°C, 30 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.7700
$Y_{DM}$: 70.57%
Yield based on 100g JA: 14.11 g
DP: 7.17
∑MU extracted: 8.694 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 22**

Description: February 05 harvested, fresh, 40 ml, 60°C, 50 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.8842
$Y_{DM}$: 78.92%
Yield based on 100g JA: 15.78 g
DP: 6.7
∑MU extracted: 9.723 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 23**

Description: February 05 harvested, fresh, 40 ml, 60°C, 60 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.8975
Yield based on 100g JA: 15.80 g
∑MU extracted: 9.730 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$: 78.98%
DP: 6.8

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 24**

Description: February 05 harvested,10 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9628
Yield based on 100g JA: 17.14 g
∑MU extracted: 10.558 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$: 85.70%
DP: 6.96

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 25**

Description: February 05 harvested,10 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, peeled

Absorbance@520nm: 0.6315
Yield based on 100g JA: 12.80 g
$Y_{DM}$: 64.00%
DP: 7.94

∑MU extracted: 7.885 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 26**

Description: February 05 harvested,10 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.9781
$Y_{DM}$: 81.03%
Yield based on 100g JA: 16.21 g
DP: 6.47
∑MU extracted: 9.982 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 27**

Description: February 05 harvested,10 day stored, 40 ml, 60°C, 40 min, WB, 13 mM acidic, Uncooked, whole

Absorbance@520nm: 0.8836
$Y_{DM}$: 76.20%
Yield based on 100g JA: 15.24 g
DP: 6.74
∑MU extracted: 9.388 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 28**

Description: February 05 harvested,10 day stored, 40 ml, 60°C, 40 min, WB, 39 mM acidic, Uncooked, whole

Absorbance@520nm: 0.9142
Yield based on 100g JA: 15.61 g
∑MU extracted: 9.613 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:78.03%
DP: 6.67

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 29**

Description: February 05 harvested,20 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9930
Yield based on 100g JA: 18.30 g
∑MU extracted: 11.274 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:91.51%
DP: 6.21

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 30**

Description: June 05 harvested,30 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.2936
Yield based on 100g JA: 7.29 g
$Y_{DM}$:36.44%
DP: 9.75

∑MU extracted: 4.489 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 31**

Description: June 05 harvested,30 day stored, 40 ml, 60°C, 40 min, WB,26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.3563
$Y_{DM}$:43.46%
Yield based on 100g JA: 8.69 g
DP: 9.58
∑MU extracted: 5.354 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 32**

Description: June 05 harvested,30 day stored, 40 ml, 60°C, 40 min, WB, NA, 20 min-cooked, whole

Absorbance@520nm: 0.5503
$Y_{DM}$:92.89%
Yield based on 100g JA: 18.58 g
DP: 13.30
∑MU extracted: 11.444 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 33**

Description: June 05 harvested,30 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, 20 min-cooked, whole

Absorbance@520nm: 0.7230
Yield based on 100g JA: 19.73 g
∑MU extracted: 12.155 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:98.66%
DP: 10.73

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 34**

Description: January 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6410
Yield based on 100g JA: 14.02 g
∑MU extracted: 8.639 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
$Y_{DM}$:70.12%
DP: 8.58

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 35**

Description: January 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6788
Yield based on 100g JA: 14.13 g
$Y_{DM}$:70.67%
DP: 8.16

∑MU extracted: 8.707 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 36**

Description: January 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6755
$Y_{DM}$:72.68%
Yield based on 100g JA: 14.54 g
DP: 8.41
∑MU extracted: 8.954 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 37**

Description: January 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.8427
$Y_{DM}$:79.57%
Yield based on 100g JA: 15.91 g
DP: 7.39
∑MU extracted: 9.803 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

## EXPERIMENT NO: 38

Description: January 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9190 $Y_{DM}$:82.64%
Yield based on 100g JA: 16.53 g DP: 7.03
∑MU extracted: 10.181 mmol
∑DP 1-2 extracted: 0.862 mmol – 8.47%
∑DP 3-4 extracted: 0.5483 mmol – 5.39%
∑DP 3-6 extracted (estimated): 0.9967 mmol – 9.79%
∑DP 1-2 in JA: 7.00%
∑DP 3-4 in JA: 4.45%
∑DP 3-6 in JA (estimated): 8.09%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.3631 | 0.0807 | 0.0807 |
| Glucose | 0.0814 | 0.0181 | 0.0181 |
| Sucrose | 3.2630 | 0.3816 | 0.7632 |
| Kestose | 0.9545 | 0.0757 | 0.2271 |
| Nystose | 1.3363 | 0.0803 | 0.3212 |
| DP5 | 0.4809 | 0.0232 | 0.1160 |
| DP6 | 1.3722 | 0.0554 | 0.3324 |

Density: 0.975 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 2.0019 ΔE: 37.92

## EXPERIMENT NO: 39

Description: January 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9492 $Y_{DM}$:84.25%
Yield based on 100g JA: 16.85 g DP: 6.94
∑MU extracted: 10.38 mmol
∑DP 1-2 extracted: 0.8852 mmol – 8.53%
∑DP 3-4 extracted: 0.7159 mmol – 6.90%
∑DP 3-6 extracted (estimated): 1.1869 mmol – 11.43%
∑DP 1-2 in JA: 7.19%

∑DP 3-4 in JA: 5.81%
∑DP 3-6 in JA (estimated): 9.63%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1793 | 0.0398 | 0.0398 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 3.6140 | 0.4227 | 0.8454 |
| Kestose | 1.4827 | 0.1177 | 0.3531 |
| Nystose | 1.5106 | 0.0907 | 0.3628 |
| DP5 | 1.0557 | 0.0510 | 0.2550 |
| DP6 | 0.8907 | 0.0360 | 0.2160 |

Density: 0.978 g/ml
Absorbance@420 nm: 2.1111
Viscosity: 1.05 cp
ΔE: 40.47

**EXPERIMENT NO: 40**

Description: January 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.7263
Yield based on 100g JA: 14.57 g
$Y_{DM}$:72.87%
DP: 7.86
∑MU extracted: 8.978 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Absorbance@420 nm: ND
Viscosity: ND
ΔE: ND

**EXPERIMENT NO: 41**

Description: January 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.8965
Yield based on 100g JA: 15.98 g
$Y_{DM}$:79.91%
DP: 6.97
∑MU extracted: 9.845 mmol
∑DP 1-2 extracted: ND

∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
Density: ND Viscosity: ND
Absorbance@420 nm: ND ΔE: ND

**EXPERIMENT NO: 42**

Description: January 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.9379 $Y_{DM}$:82.77%
Yield based on 100g JA: 16.55 g DP: 6.90
∑MU extracted: 10.2 mmol
∑DP 1-2 extracted: 0.8605 mmol – 8.44%
∑DP 3-4 extracted: 0.4243 mmol – 4.16%
∑DP 3-6 extracted (estimated): 0.9942 mmol – 9.75%
∑DP 1-2 in JA: 6.98%
∑DP 3-4 in JA: 3.44%
∑DP 3-6 in JA (estimated): 8.07%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1635 | 0.0363 | 0.0363 |
| Glucose | 0.0655 | 0.0146 | 0.0146 |
| Sucrose | 3.4607 | 0.4048 | 0.8096 |
| Kestose | 0.5308 | 0.0421 | 0.1263 |
| Nystose | 1.2409 | 0.0745 | 0.2980 |
| DP5 | 0.7455 | 0.0361 | 0.1805 |
| DP6 | 1.6058 | 0.0649 | 0.3894 |

Density: 0.977 g/ml Viscosity: 1.04 cp
Absorbance@420 nm: 0.3742 ΔE: 11.73

**EXPERIMENT NO: 43**

Description: January 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.9713 $Y_{DM}$:84.74%
Yield based on 100g JA: 16.95 g DP: 6.82
∑MU extracted: 10.440 mmol
∑DP 1-2 extracted: 0.961 mmol – 9.20%
∑DP 3-4 extracted: 1.138 mmol – 10.90%
∑DP 3-6 extracted (estimated): 1.435 mmol – 13.75%
∑DP 1-2 in JA: 7.80%
∑DP 3-4 in JA: 9.24%
∑DP 3-6 in JA (estimated): 11.65%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1527 | 0.0339 | 0.0339 |
| Glucose | 0.0463 | 0.0103 | 0.0103 |
| Sucrose | 3.9190 | 0.4584 | 0.9168 |
| Kestose | 2.7731 | 0.2200 | 0.6600 |
| Nystose | 1.9900 | 0.1195 | 0.4780 |
| DP5 | 1.0621 | 0.0510 | 0.2550 |
| DP6 | 0.1810 | 0.0070 | 0.0420 |

Density: 0.983 g/ml Viscosity: 1.15 cp
Absorbance@420 nm: 0.3761 ΔE: 12.19

**EXPERIMENT NO: 44**

Description: February 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.7664 $Y_{DM}$:72.36%
Yield based on 100g JA: 14.47 g DP: 7.39
∑MU extracted: 8.915 mmol
∑DP 1-2 extracted: 0.7018 mmol – 7.87%
∑DP 3-4 extracted: 0.529 mmol – 5.93%
∑DP 3-6 extracted (estimated): 1.124 mmol – 12.61%
∑DP 1-2 in JA: 5.70%
∑DP 3-4 in JA: 4.29%
∑DP 3-6 in JA (estimated): 9.12%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.4040 | 0.0900 | 0.0900 |
| Glucose | 0.3700 | 0.0820 | 0.0820 |

| | | | |
|---|---|---|---|
| Sucrose | 2.2640 | 0.2650 | 0.5300 |
| Kestose | 1.3480 | 0.1070 | 0.3210 |
| Nystose | 0.8720 | 0.0520 | 0.2080 |
| DP5 | 1.4800 | 0.0710 | 0.3550 |
| DP6 | 0.9920 | 0.0400 | 0.2400 |

Density: 0.962 g/ml Viscosity: 1.00 cp
Absorbance@420 nm: 1.8518 ΔE: 37.06

**EXPERIMENT NO: 45**

Description: February 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.7988 $Y_{DM}$:73.01%
Yield based on 100g JA: 14.60 g DP: 7.15
∑MU extracted: 8.995 mmol
∑DP 1-2 extracted: 0.818 mmol – 9.09%
∑DP 3-4 extracted: 0.779 mmol – 8.66%
∑DP 3-6 extracted (estimated): 1.372 mmol – 15.25%
∑DP 1-2 in JA: 6.64%
∑DP 3-4 in JA: 6.32%
∑DP 3-6 in JA (estimated): 11.14%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2900 | 0.0640 | 0.0640 |
| Glucose | 0.7020 | 0.1560 | 0.1560 |
| Sucrose | 2.5570 | 0.2990 | 0.5980 |
| Kestose | 1.7780 | 0.1410 | 0.4230 |
| Nystose | 1.4850 | 0.0890 | 0.3560 |
| DP5 | 1.2710 | 0.0610 | 0.3050 |
| DP6 | 1.1850 | 0.0480 | 0.2880 |

Density: 0.966 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 0.3945 ΔE: 13.69

## EXPERIMENT NO: 46

Description: February 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9060 $Y_{DM}$:82.01%
Yield based on 100g JA: 16.40 g DP: 7.08
∑MU extracted: 10.104 mmol
∑DP 1-2 extracted: 0.843 mmol – 8.34%
∑DP 3-4 extracted: 0.679 mmol – 6.72%
∑DP 3-6 extracted (estimated): 1.387 mmol – 13.73%
∑DP 1-2 in JA: 6.84%
∑DP 3-4 in JA: 5.51%
∑DP 3-6 in JA (estimated): 11.26%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.8190 | 0.1820 | 0.1820 |
| Glucose | 0.6705 | 0.1490 | 0.1490 |
| Sucrose | 2.1713 | 0.2560 | 0.5120 |
| Kestose | 2.6823 | 0.2130 | 0.6390 |
| Nystose | 0.1643 | 0.0100 | 0.0400 |
| DP5 | 1.5000 | 0.0720 | 0.3600 |
| DP6 | 1.4265 | 0.0580 | 0.3480 |

Density: 0.966 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.8710 ΔE: 38.15

## EXPERIMENT NO: 47

Description: February 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9457 $Y_{DM}$:82.63%
Yield based on 100g JA: 16.53 g DP: 6.83
∑MU extracted: 10.180 mmol
∑DP 1-2 extracted: 0.699 mmol – 6.87%
∑DP 3-4 extracted: 0.588 mmol – 5.78%
∑DP 3-6 extracted (estimated): 1.229 mmol – 12.07%
∑DP 1-2 in JA: 5.67%
∑DP 3-4 in JA: 4.77%
∑DP 3-6 in JA (estimated): 9.98%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1515 | 0.1340 | 0.1340 |
| Glucose | 0.0630 | 0.0190 | 0.0190 |
| Sucrose | 2.3322 | 0.2730 | 0.5460 |
| Kestose | 1.2034 | 0.0960 | 0.2880 |
| Nystose | 1.2420 | 0.0750 | 0.3000 |
| DP5 | 1.1470 | 0.0550 | 0.2750 |
| DP6 | 1.5080 | 0.0610 | 0.3660 |

Density: 0.974 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 1.9439 ΔE: 38.52

**EXPERIMENT NO: 48**

Description: February 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.9808 $Y_{DM}$:83.72%
Yield based on 100g JA: 16.74 g DP: 6.67
∑MU extracted: 10.314 mmol
∑DP 1-2 extracted: 1.453 mmol – 14.09%
∑DP 3-4 extracted: 0.637 mmol – 6.18%
∑DP 3-6 extracted (estimated): 1.503 mmol – 14.57%
∑DP 1-2 in JA: 11.79%
∑DP 3-4 in JA: 5.17%
∑DP 3-6 in JA (estimated): 12.20%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 3.8655 | 0.8590 | 0.8590 |
| Glucose | 2.6730 | 0.5940 | 0.5940 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 1.2435 | 0.0990 | 0.2970 |
| Nystose | 1.4070 | 0.0850 | 0.3400 |
| DP5 | 2.0790 | 0.1000 | 0.5000 |
| DP6 | 1.5020 | 0.0610 | 0.3660 |

Density: 0.979 g/ml Viscosity: 1.05 cp
Absorbance@420 nm: 2.1311 ΔE: 42.39

**EXPERIMENT NO: 49**

Description: February 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 1.0268 $Y_{DM}$:84.28%
Yield based on 100g JA: 16.86 g DP: 6.41
∑MU extracted: 10.383 mmol
∑DP 1-2 extracted: 0.978 mmol – 9.42%
∑DP 3-4 extracted: 1.331 mmol – 12.82%
∑DP 3-6 extracted (estimated): 2.074 mmol – 19.97%
∑DP 1-2 in JA: 7.94%
∑DP 3-4 in JA: 10.80%
∑DP 3-6 in JA (estimated): 16.83%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.5860 | 0.1300 | 0.1300 |
| Glucose | 0.0460 | 0.0100 | 0.0100 |
| Sucrose | 3.5860 | 0.4190 | 0.8380 |
| Kestose | 3.1835 | 0.2530 | 0.7590 |
| Nystose | 2.3770 | 0.1430 | 0.5720 |
| DP5 | 1.6380 | 0.0790 | 0.3950 |
| DP6 | 1.4330 | 0.0580 | 0.3480 |

Density: 0.981 g/ml Viscosity: 1.08 cp
Absorbance@420 nm: 2.2271 ΔE: 45.74

**EXPERIMENT NO: 50**

Description: February 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.9269 $Y_{DM}$: 82.38%
Yield based on 100g JA: 16.48 g DP: 6.95
∑MU extracted: 10.150 mmol
∑DP 1-2 extracted: 0.953 mmol – 9.39%
∑DP 3-4 extracted: 1.037 mmol – 10.22%
∑DP 3-6 extracted (estimated): 1.714 mmol – 16.89%
∑DP 1-2 in JA: 7.74%
∑DP 3-4 in JA: 8.42%

∑DP 3-6 in JA (estimated): 13.91%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.5895 | 0.1310 | 0.1310 |
| Glucose | 0.8820 | 0.1960 | 0.1960 |
| Sucrose | 2.6762 | 0.3130 | 0.6260 |
| Kestose | 2.6025 | 0.2070 | 0.6210 |
| Nystose | 1.7270 | 0.1040 | 0.4160 |
| DP5 | 1.2860 | 0.0670 | 0.3350 |
| DP6 | 1.4100 | 0.0570 | 0.3420 |

Density: 0.968 g/ml Viscosity: 1.04 cp
Absorbance@420 nm: 0.3952 ΔE: 13.73

**EXPERIMENT NO: 51**

Description: February 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.968 $Y_{DM}$: 82.87%
Yield based on 100g JA: 16.57 g DP: 6.69
∑MU extracted: 10.210 mmol
∑DP 1-2 extracted: 0.935 mmol – 9.16%
∑DP 3-4 extracted: 1.041 mmol – 10.20%
∑DP 3-6 extracted (estimated): 1.729 mmol – 16.93%
∑DP 1-2 in JA: 7.59%
∑DP 3-4 in JA: 8.45%
∑DP 3-6 in JA (estimated): 14.03%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2530 | 0.1570 | 0.1570 |
| Glucose | 0.0480 | 0.1740 | 0.1740 |
| Sucrose | 2.5790 | 0.3020 | 0.6040 |
| Kestose | 2.7580 | 0.2190 | 0.6570 |
| Nystose | 1.6020 | 0.0960 | 0.3840 |
| DP5 | 1.4160 | 0.0680 | 0.3400 |
| DP6 | 1.4450 | 0.0580 | 0.3480 |

Density: 0.984 g/ml Viscosity: 1.05 cp

Absorbance@420 nm: 0.3956 ΔE: 13.78

**EXPERIMENT NO: 52**

Description: February 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.9881 $Y_{DM}$:83.97%
Yield based on 100g JA: 16.79 g DP: 6.64
∑MU extracted: 10.345 mmol
∑DP 1-2 extracted: 0.91 mmol – 8.80%
∑DP 3-4 extracted: 1.104 mmol – 10.67%
∑DP 3-6 extracted (estimated): 1.939 mmol – 18.74%
∑DP 1-2 in JA: 7.39%
∑DP 3-4 in JA: 8.96%
∑DP 3-6 in JA (estimated): 15.74%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1840 | 0.1410 | 0.1410 |
| Glucose | 0.0380 | 0.1390 | 0.1390 |
| Sucrose | 2.6935 | 0.3150 | 0.6300 |
| Kestose | 2.2652 | 0.2320 | 0.6960 |
| Nystose | 1.7040 | 0.1020 | 0.4080 |
| DP5 | 1.3900 | 0.0710 | 0.3550 |
| DP6 | 1.9910 | 0.0800 | 0.4800 |

Density: 0.986 g/ml Viscosity: 1.08 cp
Absorbance@420 nm: 0.3968 ΔE: 13.94

**EXPERIMENT NO: 53**

Description: February 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 1.0595 $Y_{DM}$:84.70%
Yield based on 100g JA: 16.94 g DP: 6.24
∑MU extracted: 10.435 mmol
∑DP 1-2 extracted: 1.05 mmol – 10.06%
∑DP 3-4 extracted: 1.507 mmol – 14.44%

∑DP 3-6 extracted (estimated): 2.446 mmol – 23.44%
∑DP 1-2 in JA: 8.52%
∑DP 3-4 in JA: 12.23%
∑DP 3-6 in JA (estimated): 19.85%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.3470 | 0.0770 | 0.0770 |
| Glucose | 0.2280 | 0.0510 | 0.0510 |
| Sucrose | 3.9450 | 0.4610 | 0.9220 |
| Kestose | 3.6935 | 0.2930 | 0.8790 |
| Nystose | 2.6235 | 0.1570 | 0.6280 |
| DP5 | 1.9330 | 0.0930 | 0.4650 |
| DP6 | 1.9530 | 0.0790 | 0.4740 |

Density: 0.988 g/ml
Viscosity: 1.46 cp
Absorbance@420 nm: 0.3975
ΔE: 13.98

**EXPERIMENT NO: 54**

Description: February 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, 1 min-cooked, whole

Absorbance@520nm: 0.8312
$Y_{DM}$:95.43%
Yield based on 100g JA: 19.09 g
DP: 9.01
∑MU extracted: 11.76 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 55**

Description: February 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, 26 mM acidic, 1 min-cooked, whole

Absorbance@520nm: 1.0507 $Y_{DM}$:99.60%
Yield based on 100g JA: 19.92 g DP: 7.42
∑MU extracted: 12.270 mmol
∑DP 1-2 extracted: 0.709 mmol – 5.78%
∑DP 3-4 extracted: 0.665 mmol – 5.42%
∑DP 3-6 extracted (estimated): 1.881 mmol – 15.33%
∑DP 1-2 in JA: 5.75%
∑DP 3-4 in JA: 5.40%
∑DP 3-6 in JA (estimated): 15.27%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1485 | 0.0330 | 0.0330 |
| Glucose | 0.9180 | 0.2040 | 0.2040 |
| Sucrose | 2.0140 | 0.2360 | 0.4720 |
| Kestose | 1.5540 | 0.1230 | 0.3690 |
| Nystose | 1.2390 | 0.0740 | 0.2960 |
| DP5 | 3.6396 | 0.1760 | 0.8800 |
| DP6 | 1.3905 | 0.0560 | 0.3360 |

Density: 0.981 g/ml Viscosity: 1.16 cp
Absorbance@420 nm: 1.1984 ΔE: 34.16

**EXPERIMENT NO: 56**

Description: March 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6347 $Y_{DM}$:70.00%
Yield based on 100g JA: 14.00 g DP: 8.65
∑MU extracted: 8.624 mmol
∑DP 1-2 extracted: 1.052 mmol – 12.20%
∑DP 3-4 extracted: 1.0977 mmol – 12.73%
∑DP 3-6 extracted (estimated): 1.2859mmol – 14.91%
∑DP 1-2 in JA: 8.54%
∑DP 3-4 in JA: 8.91%
∑DP 3-6 in JA (estimated): 10.44%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 3.0326 | 0.6740 | 0.6740 |
| Glucose | 0.0698 | 0.0160 | 0.0160 |
| Sucrose | 1.5473 | 0.1810 | 0.3620 |
| Kestose | 1.3494 | 0.1071 | 0.3213 |
| Nystose | 3.2315 | 0.1941 | 0.7764 |
| DP5 | 0.3639 | 0.0176 | 0.0880 |
| DP6 | 0.4140 | 0.0167 | 0.1002 |

Density: 0.959 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.7503 ΔE: 40.92

**EXPERIMENT NO: 57**

Description: March 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6512 $Y_{DM}$:70.42%
Yield based on 100g JA: 14.08 g DP: 8.48
∑MU extracted: 8.676 mmol
∑DP 1-2 extracted: 0.7117 mmol – 8.20%
∑DP 3-4 extracted: 1.4668 mmol – 16.91%
∑DP 3-6 extracted (estimated): 1.5782 mmol – 18.19%
∑DP 1-2 in JA: 5.78%
∑DP 3-4 in JA: 11.91%
∑DP 3-6 in JA (estimated): 12.81%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1839 | 0.0409 | 0.0409 |
| Glucose | 0.0685 | 0.0152 | 0.0152 |
| Sucrose | 2.8026 | 0.3278 | 0.6556 |
| Kestose | 3.8709 | 0.3072 | 0.9216 |
| Nystose | 2.2690 | 0.1363 | 0.5452 |
| DP5 | 0.2773 | 0.0134 | 0.0670 |
| DP6 | 0.1842 | 0.0074 | 0.0440 |

Density: 0.964 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 0.3708 ΔE: 13.02

**EXPERIMENT NO: 58**

Description: March 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6474 $Y_{DM}$:70.42%
Yield based on 100g JA: 14.08 g DP: 8.53
∑MU extracted: 8.676 mmol
∑DP 1-2 extracted: 0.8022 mmol – 9.25%
∑DP 3-4 extracted: 1.2188 mmol – 14.05%
∑DP 3-6 extracted (estimated): 1.4872 mmol – 17.14%
∑DP 1-2 in JA: 6.51%
∑DP 3-4 in JA: 9.89%
∑DP 3-6 in JA (estimated): 12.07%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.4489 | 0.0997 | 0.0997 |
| Glucose | 0.1059 | 0.0235 | 0.0235 |
| Sucrose | 2.9030 | 0.3395 | 0.6790 |
| Kestose | 3.2607 | 0.2588 | 0.7764 |
| Nystose | 1.8418 | 0.1106 | 0.4424 |
| DP5 | 1.0399 | 0.0502 | 0.2510 |
| DP6 | 0.0702 | 0.0029 | 0.0174 |

Density: 0.965 g/ml Viscosity: 1.02cp
Absorbance@420 nm: 1.9713 ΔE: 41.54

**EXPERIMENT NO: 59**

Description: March 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.7386 $Y_{DM}$:74.11%
Yield based on 100g JA: 14.82 g DP: 7.86
∑MU extracted: 9.130 mmol
∑DP 1-2 extracted: 1.1579 mmol – 12.68%
∑DP 3-4 extracted: 1.1342 mmol – 12.42%
∑DP 3-6 extracted (estimated): 1.3792 mmol – 15.11%
∑DP 1-2 in JA: 9.40%

∑DP 3-4 in JA: 9.21%
∑DP 3-6 in JA (estimated): 11.19%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1620 | 0.0360 | 0.0360 |
| Glucose | 3.6841 | 0.8187 | 0.8187 |
| Sucrose | 1.2964 | 0.1516 | 0.3032 |
| Kestose | 0.9700 | 0.0770 | 0.2310 |
| Nystose | 3.7597 | 0.2258 | 0.9032 |
| DP5 | 0.7662 | 0.0370 | 0.1850 |
| DP6 | 0.2477 | 0.0100 | 0.0600 |

Density: 0.969 g/ml Viscosity: 1.04 cp
Absorbance@420 nm: 1.9799 ΔE: 41.72

**EXPERIMENT NO: 60**

Description: March 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.7821 $Y_{DM}$:75.03%
Yield based on 100g JA: 15.01 g DP: 7.51
∑MU extracted: 9.244 mmol
∑DP 1-2 extracted: 0.8594 mmol – 9.30%
∑DP 3-4 extracted: 0.9164 mmol – 9.91%
∑DP 3-6 extracted (estimated): 1.3198 mmol – 14.28%
∑DP 1-2 in JA: 6.98%
∑DP 3-4 in JA: 7.44%
∑DP 3-6 in JA (estimated): 10.71%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.4382 | 0.0974 | 0.0974 |
| Glucose | 0.8019 | 0.1782 | 0.1782 |
| Sucrose | 2.4957 | 0.2919 | 0.5838 |
| Kestose | 1.5575 | 0.1236 | 0.3708 |
| Nystose | 2.2706 | 0.1364 | 0.5456 |
| DP5 | 1.0754 | 0.0520 | 0.2600 |
| DP6 | 0.5921 | 0.0239 | 0.1434 |

Density: 0.974 g/ml Viscosity: 1.04 cp

Absorbance@420 nm: 1.9897 ΔE: 42.69

**EXPERIMENT NO: 61**

Description: March 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.8376 $Y_{DM}$:76.77%
Yield based on 100g JA: 15.35 g DP: 7.17
∑MU extracted: 9.458 mmol
∑DP 1-2 extracted: 0.9205 mmol – 9.73%
∑DP 3-4 extracted: 0.7955 mmol – 8.41%
∑DP 3-6 extracted (estimated): 1.3236 mmol – 13.99%
∑DP 1-2 in JA: 7.47%
∑DP 3-4 in JA: 6.46%
∑DP 3-6 in JA (estimated): 10.74%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.0295 | 0.2288 | 0.2288 |
| Glucose | 0.8978 | 0.1995 | 0.1995 |
| Sucrose | 2.1042 | 0.2461 | 0.4922 |
| Kestose | 2.5718 | 0.2041 | 0.6123 |
| Nystose | 0.7623 | 0.0458 | 0.1832 |
| DP5 | 1.3516 | 0.0653 | 0.3265 |
| DP6 | 0.8327 | 0.0336 | 0.2016 |

Density: 0.977 g/ml Viscosity: 1.05 cp
Absorbance@420 nm: 1.9916 ΔE: 45.41

**EXPERIMENT NO: 62**

Description: March 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6793 $Y_{DM}$:70.81%
Yield based on 100g JA: 14.16 g DP: 8.17
∑MU extracted: 8.724 mmol

∑DP 1-2 extracted: 0.8534 mmol – 9.78%
∑DP 3-4 extracted: 0.6848 mmol – 7.85%
∑DP 3-6 extracted (estimated): 1.2561 mmol – 14.40%
∑DP 1-2 in JA: 6.93%
∑DP 3-4 in JA: 5.56%
∑DP 3-6 in JA (estimated): 10.20%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.3428 | 0.2984 | 0.2984 |
| Glucose | 0.1540 | 0.0342 | 0.0342 |
| Sucrose | 2.2266 | 0.2604 | 0.5208 |
| Kestose | 1.4719 | 0.1168 | 0.3504 |
| Nystose | 1.3919 | 0.0836 | 0.3344 |
| DP5 | 0.9429 | 0.0455 | 0.2275 |
| DP6 | 1.4183 | 0.0573 | 0.3438 |

Density: 0.966 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 0.3727 ΔE: 13.23

**EXPERIMENT NO: 63**

Description: March 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.7654 $Y_{DM}$:74.87%
Yield based on 100g JA: 14.97 g DP: 7.66
∑MU extracted: 9.224 mmol
∑DP 1-2 extracted: 1.0029 mmol – 10.87%
∑DP 3-4 extracted: 0.7655 mmol – 8.30%
∑DP 3-6 extracted (estimated): 1.3827 mmol – 14.99%
∑DP 1-2 in JA: 8.14%
∑DP 3-4 in JA: 6.21%
∑DP 3-6 in JA (estimated): 11.22%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.3563 | 0.3014 | 0.3014 |
| Glucose | 1.0516 | 0.2337 | 0.2337 |
| Sucrose | 2.0000 | 0.2339 | 0.4678 |
| Kestose | 1.6745 | 0.1329 | 0.3987 |
| Nystose | 1.5277 | 0.0917 | 0.3668 |
| DP5 | 1.1846 | 0.0572 | 0.2860 |
| DP6 | 1.3661 | 0.0552 | 0.3312 |

Density: 0.975 g/ml Viscosity: 1.05 cp
Absorbance@420 nm: 0.3732 ΔE: 13.49

**EXPERIMENT NO: 64**

Description: March 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.8075 $Y_{DM}$:75.53%
Yield based on 100g JA: 15.11 g DP: 7.32
∑MU extracted: 9.305 mmol
∑DP 1-2 extracted: 0.9016 mmol – 9.69%
∑DP 3-4 extracted: 0.7966 mmol – 8.56%
∑DP 3-6 extracted (estimated): 1.4109 mmol – 15.16%
∑DP 1-2 in JA: 7.32%
∑DP 3-4 in JA: 6.47%
∑DP 3-6 in JA (estimated): 11.45%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.1070 | 0.2460 | 0.2460 |
| Glucose | 0.3436 | 0.0764 | 0.0764 |
| Sucrose | 2.4758 | 0.2896 | 0.5792 |
| Kestose | 2.0080 | 0.1594 | 0.4782 |
| Nystose | 1.3251 | 0.0796 | 0.3184 |
| DP5 | 1.1946 | 0.0577 | 0.2885 |
| DP6 | 1.3448 | 0.0543 | 0.3258 |

Density: 0.978 g/ml Viscosity: 1.06 cp
Absorbance@420 nm: 0.3761 ΔE: 13.64

**EXPERIMENT NO: 65**

Description: March 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.8613 $Y_{DM}$:76.77%
Yield based on 100g JA: 15.35 g DP: 6.97

∑MU extracted: 9.458 mmol
∑DP 1-2 extracted: 0.8872 mmol – 9.38%
∑DP 3-4 extracted: 0.9166 mmol – 9.69%
∑DP 3-6 extracted (estimated): 1.4904 mmol – 15.76%
∑DP 1-2 in JA: 7.20%
∑DP 3-4 in JA: 7.44%
∑DP 3-6 in JA (estimated): 12.10%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2352 | 0.0523 | 0.0523 |
| Glucose | 1.0084 | 0.2241 | 0.2241 |
| Sucrose | 2.6114 | 0.3054 | 0.6108 |
| Kestose | 2.1444 | 0.1702 | 0.5106 |
| Nystose | 1.6904 | 0.1015 | 0.4060 |
| DP5 | 1.6850 | 0.0814 | 0.4070 |
| DP6 | 0.6882 | 0.0278 | 0.1668 |

Density: 0.984 g/ml Viscosity: 1.27 cp
Absorbance@420 nm: 0.3790 ΔE: 13.79

**EXPERIMENT NO: 66**

Description: April 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.5781 $Y_{DM}$:65.61%
Yield based on 100g JA: 13.12 g DP: 9.00
∑MU extracted: 8.083 mmol
∑DP 1-2 extracted: 0.6693 mmol – 8.28%
∑DP 3-4 extracted: 0.904 mmol – 11.18%
∑DP 3-6 extracted (estimated): 1.0652 mmol – 13.18%
∑DP 1-2 in JA: 5.43%
∑DP 3-4 in JA: 7.34%
∑DP 3-6 in JA (estimated): 8.65%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1530 | 0.0340 | 0.0340 |
| Glucose | 0.1590 | 0.0353 | 0.0353 |
| Sucrose | 2.5610 | 0.3000 | 0.6000 |
| Kestose | 2.3190 | 0.1840 | 0.5520 |
| Nystose | 1.4720 | 0.0880 | 0.3520 |
| DP5 | 0.4086 | 0.0200 | 0.1000 |
| DP6 | 0.2527 | 0.0102 | 0.0612 |

Density: 0.958 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.7027 ΔE: 43.52

**EXPERIMENT NO: 67**

Description: April 06 harvested, fresh, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6107 $Y_{DM}$:67.35%
Yield based on 100g JA: 13.47 g DP: 8.65
∑MU extracted: 8.300 mmol
∑DP 1-2 extracted: 0.592 mmol – 7.13%
∑DP 3-4 extracted: 0.926 mmol – 11.16%
∑DP 3-6 extracted (estimated): 1.077 mmol – 12.98%
∑DP 1-2 in JA: 4.81%
∑DP 3-4 in JA: 7.52%
∑DP 3-6 in JA (estimated): 8.74%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | - | 0 |
| Glucose | 0 | - | 0 |
| Sucrose | 2.5350 | 0.2960 | 0.5920 |
| Kestose | 2.1910 | 0.1740 | 0.5220 |
| Nystose | 1.6770 | 0.1010 | 0.4040 |
| DP5 | 0.1116 | 0.0050 | 0.0250 |
| DP6 | 0.5197 | 0.0210 | 0.1260 |

Density: 0.963 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 0.3549 ΔE: 12.53

**EXPERIMENT NO: 68**

Description: April 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.5996 $Y_{DM}$:66.80%
Yield based on 100g JA: 13.36 g DP: 8.74

∑MU extracted: 8.230 mmol
∑DP 1-2 extracted: 0.6546 mmol – 7.95%
∑DP 3-4 extracted: 0.9202 mmol – 11.18%
∑DP 3-6 extracted (estimated): 1.1264 mmol – 13.69%
∑DP 1-2 in JA: 5.31%
∑DP 3-4 in JA: 7.47%
∑DP 3-6 in JA (estimated): 9.14%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2680 | 0.0600 | 0.0600 |
| Glucose | 0.3370 | 0.0750 | 0.0750 |
| Sucrose | 2.2210 | 0.2598 | 0.5196 |
| Kestose | 3.8030 | 0.3018 | 0.9054 |
| Nystose | 0.0620 | 0.0037 | 0.0148 |
| DP5 | 0.7130 | 0.0344 | 0.1720 |
| DP6 | 0.1425 | 0.0057 | 0.0342 |

Density: 0.964 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 1.7069 ΔE: 43.61

**EXPERIMENT NO: 69**

Description: April 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6299 $Y_{DM}$:68.35%
Yield based on 100g JA: 13.67 g DP: 8.51
∑MU extracted: 8.421 mmol
∑DP 1-2 extracted: 0.964 mmol – 11.45%
∑DP 3-4 extracted: 0.7654 mmol – 9.09%
∑DP 3-6 extracted (estimated): 0.952 mmol – 11.31%
∑DP 1-2 in JA: 7.82%
∑DP 3-4 in JA: 6.21%
∑DP 3-6 in JA (estimated): 7.73%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.4100 | 0.3130 | 0.3130 |
| Glucose | 2.9295 | 0.6510 | 0.6510 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 1.1265 | 0.0894 | 0.2682 |
| Nystose | 2.0690 | 0.1243 | 0.4972 |
| DP5 | 0.3735 | 0.0180 | 0.0900 |
| DP6 | 0.3983 | 0.0161 | 0.0966 |

Density: 0.967 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 1.7098 ΔE: 44.04

**EXPERIMENT NO: 70**

Description: April 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6575 $Y_{DM}$:70.11%
Yield based on 100g JA: 14.02 g DP: 8.36
∑MU extracted: 8.640 mmol
∑DP 1-2 extracted: 0.5551 mmol – 6.42%
∑DP 3-4 extracted: 0.7834 mmol – 9.07%
∑DP 3-6 extracted (estimated): 0.9962 mmol – 11.53%
∑DP 1-2 in JA: 4.51%
∑DP 3-4 in JA: 6.36%
∑DP 3-6 in JA (estimated): 8.09%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.3630 | 0.0807 | 0.0807 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 2.0280 | 0.2372 | 0.4744 |
| Kestose | 1.8825 | 0.1494 | 0.4482 |
| Nystose | 1.3961 | 0.0838 | 0.3352 |
| DP5 | 0.1044 | 0.0050 | 0.0250 |
| DP6 | 0.7740 | 0.0313 | 0.1878 |

Density: 0.973 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 1.7123 ΔE: 44.29

**EXPERIMENT NO: 71**

Description: April 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.6810 $Y_{DM}$: 72.02%
Yield based on 100g JA: 14.40 g DP: 8.29

∑MU extracted: 8.873 mmol
∑DP 1-2 extracted: 0.5768 mmol – 6.50%
∑DP 3-4 extracted: 1.0052 mmol – 11.33%
∑DP 3-6 extracted (estimated): 1.198 mmol – 13.50%
∑DP 1-2 in JA: 4.68%
∑DP 3-4 in JA: 8.16%
∑DP 3-6 in JA (estimated): 9.72%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | - | 0 |
| Glucose | 0 | - | 0 |
| Sucrose | 2.4660 | 0.2884 | 0.5768 |
| Kestose | 2.2785 | 0.1808 | 0.5424 |
| Nystose | 1.9271 | 0.1157 | 0.4628 |
| DP5 | 0.4581 | 0.0220 | 0.1100 |
| DP6 | 0.3427 | 0.0138 | 0.0828 |

Density: 0.974 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 1.7349 ΔE: 44.56

**EXPERIMENT NO: 72**

Description: April 06 harvested, 5 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6488 $Y_{DM}$:69.02%
Yield based on 100g JA: 13.80 g DP: 8.34
∑MU extracted: 8.503 mmol
∑DP 1-2 extracted: 0.642 mmol – 7.55%
∑DP 3-4 extracted: 0.7428 mmol – 8.74%
∑DP 3-6 extracted (estimated): 1.0458 mmol – 12.30%
∑DP 1-2 in JA: 5.21%
∑DP 3-4 in JA: 6.03%
∑DP 3-6 in JA (estimated): 8.49%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2775 | 0.0620 | 0.0620 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 2.4833 | 0.2900 | 0.5800 |
| Kestose | 1.3917 | 0.1104 | 0.3312 |
| Nystose | 1.7134 | 0.1029 | 0.4116 |
| DP5 | 0.6453 | 0.0312 | 0.1560 |
| DP6 | 0.6077 | 0.0245 | 0.1470 |

Density: 0.965 g/ml Viscosity: 1.03 cp
Absorbance@420 nm: 0.3768 ΔE: 12.62

**EXPERIMENT NO: 73**

Description: April 06 harvested, 10 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6591 $Y_{DM}$:69.62%
Yield based on 100g JA: 13.92 g DP: 8.28
∑MU extracted: 8.577 mmol
∑DP 1-2 extracted: 0.6096 mmol – 7.11%
∑DP 3-4 extracted: 0.711 mmol – 8.29%
∑DP 3-6 extracted (estimated): 1.0323 mmol – 12.04%
∑DP 1-2 in JA: 4.95%
∑DP 3-4 in JA: 5.77%
∑DP 3-6 in JA (estimated): 8.38%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2928 | 0.0650 | 0.0650 |
| Glucose | 0.1531 | 0.0340 | 0.0340 |
| Sucrose | 2.1829 | 0.2553 | 0.5106 |
| Kestose | 1.3773 | 0.1093 | 0.3279 |
| Nystose | 1.5957 | 0.0958 | 0.3832 |
| DP5 | 0.5787 | 0.0280 | 0.1400 |
| DP6 | 0.7473 | 0.0302 | 0.1812 |

Density: 0.970 g/ml Viscosity: 1.04 cp
Absorbance@420 nm: 0.3779 ΔE: 13.17

**EXPERIMENT NO: 74**

Description: April 06 harvested, 15 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.6971 $Y_{DM}$:71.88%
Yield based on 100g JA: 14.38 g DP: 8.08

∑MU extracted: 8.856 mmol
∑DP 1-2 extracted: 0.7062 mmol – 7.97%
∑DP 3-4 extracted: 0.5123 mmol – 5.78%
∑DP 3-6 extracted (estimated): 0.9786 mmol – 11.05%
∑DP 1-2 in JA: 5.73%
∑DP 3-4 in JA: 4.16%
∑DP 3-6 in JA (estimated): 7.94%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.4310 | 0.3180 | 0.3180 |
| Glucose | 0.0641 | 0.0140 | 0.0140 |
| Sucrose | 1.6000 | 0.1871 | 0.3742 |
| Kestose | 1.1403 | 0.0905 | 0.2715 |
| Nystose | 1.0024 | 0.0602 | 0.2408 |
| DP5 | 0.9920 | 0.0479 | 0.2395 |
| DP6 | 0.9350 | 0.0378 | 0.2268 |

Density: 0.975 g/ml Viscosity: 1.05 cp
Absorbance@420 nm: 0.3784 ΔE: 13.24

**EXPERIMENT NO: 75**

Description: April 06 harvested, 20 day stored, 40 ml, 60°C, 40 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 0.7047 $Y_{DM}$:72.22%
Yield based on 100g JA: 14.44 g DP: 8.03
∑MU extracted: 8.898 mmol
∑DP 1-2 extracted: 0.6528 mmol – 7.34%
∑DP 3-4 extracted: 1.151 mmol – 12.94%
∑DP 3-6 extracted (estimated): 1.3098 mmol – 14.72%
∑DP 1-2 in JA: 5.30%
∑DP 3-4 in JA: 9.34%
∑DP 3-6 in JA (estimated): 10.63%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.4840 | 0.1080 | 0.1080 |
| Glucose | 0.0765 | 0.0170 | 0.0170 |
| Sucrose | 2.2567 | 0.2639 | 0.5278 |
| Kestose | 2.8750 | 0.2282 | 0.6846 |
| Nystose | 1.9415 | 0.1166 | 0.4664 |
| DP5 | 0.3141 | 0.0152 | 0.0760 |
| DP6 | 0.3420 | 0.0138 | 0.0828 |

Density: 0.980 g/ml Viscosity: 1.25 cp
Absorbance@420 nm: 0.3802 ΔE: 13.59

**EXPERIMENT NO: 76**

Description: April 06 harvested, fresh, 40 ml, 20°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: 0.0496 $Y_{DM}$:16.61%
Yield based on 100g JA: 3.32 g DP: 26.50
∑MU extracted: 2.046 mmol
∑DP 1-2 extracted: 0.241 mmol – 11.78%
∑DP 3-4 extracted: 0.349 mmol – 17.06%
∑DP 3-6 extracted (estimated): 0.428 mmol – 20.92%
∑DP 1-2 in JA: 1.96%
∑DP 3-4 in JA: 2.83%
∑DP 3-6 in JA (estimated): 3.47%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.0835 | 0.2410 | 0.2410 |
| Glucose | 0 | - | - |
| Sucrose | 0 | - | - |
| Kestose | 0.5407 | 0.0430 | 0.1290 |
| Nystose | 0.9172 | 0.0550 | 0.2200 |
| DP5 | 0.1134 | 0.0050 | 0.0250 |
| DP6 | 0.2333 | 0.0090 | 0.0540 |

Density: 0.974 g/ml Viscosity: 0.99 cp
Absorbance@420 nm: 1.400 ΔE: 36.99

**EXPERIMENT NO: 77**

Description: April 06 harvested, fresh, 40 ml, 20°C, 3.5 min, WB, NA, Uncooked, whole

Absorbance@520nm: 0.1116 $Y_{DM}$:20.72%
Yield based on 100g JA: 4.14 g DP: 14.63

∑MU extracted: 2.552 mmol
∑DP 1-2 extracted: 0.4255 mmol – 16.67%
∑DP 3-4 extracted: 0.388 mmol – 15.20%
∑DP 3-6 extracted (estimated): 0.41 mmol – 16.07%
∑DP 1-2 in JA: 3.45%
∑DP 3-4 in JA: 3.15%
∑DP 3-6 in JA (estimated): 3.33%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.8034 | 0.1785 | 0.1785 |
| Glucose | 0.2204 | 0.0490 | 0.0490 |
| Sucrose | 0.8443 | 0.0990 | 0.1980 |
| Kestose | 0.9617 | 0.0760 | 0.2280 |
| Nystose | 0.6620 | 0.0400 | 0.1600 |
| DP5 | 0.0432 | 0.0020 | 0.0100 |
| DP6 | 0.0610 | 0.0020 | 0.0120 |

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 78**

Description: April 06 harvested, fresh, 40 ml, 20°C, 3.5 min, UB + WB, NA, Uncooked, whole

Absorbance@520nm: 0.053
$Y_{DM}$:17.23%
Yield based on 100g JA: 3.45 g
DP: 25.71
∑MU extracted: 2.123 mmol
∑DP 1-2 extracted: 0.38 mmol – 17.90%
∑DP 3-4 extracted: 0.292 mmol – 13.75%
∑DP 3-6 extracted (estimated): 0.381 mmol – 17.95%
∑DP 1-2 in JA: 3.08%
∑DP 3-4 in JA: 2.37%
∑DP 3-6 in JA (estimated): 3.09%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.0810 | 0.2400 | 0.2400 |
| Glucose | 0.6290 | 0.1400 | 0.1400 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 0 | 0 | 0 |
| Nystose | 1.2195 | 0.0730 | 0.2920 |
| DP5 | 0.2718 | 0.0130 | 0.0650 |
| DP6 | 0.1100 | 0.0040 | 0.0240 |

Density: ND Viscosity: ND
Absorbance@420 nm: ND ΔE: ND

**EXPERIMENT NO: 79**

Description: April 06 harvested, fresh, 40 ml, 40°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:22.49%
Yield based on 100g JA: 4.50 g DP: ND
∑MU extracted: 2.771 mmol
∑DP 1-2 extracted: 0.34 mmol – 12.27%
∑DP 3-4 extracted: 0.244 mmol – 8.81%
∑DP 3-6 extracted (estimated): 0.507 mmol – 18.30%
∑DP 1-2 in JA: 2.76%
∑DP 3-4 in JA: 1.98%
∑DP 3-6 in JA (estimated): 4.12%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.5300 | 0.3400 | 0.3400 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 0 | 0 | 0 |
| Nystose | 1.0160 | 0.0610 | 0.2440 |
| DP5 | 0.7720 | 0.0370 | 0.1850 |
| DP6 | 0.3285 | 0.0130 | 0.0780 |

Density: 0.976 g/ml Viscosity: 1.00 cp
Absorbance@420 nm: 1.5206 ΔE: 37.08

**EXPERIMENT NO: 80**

Description: April 06 harvested, fresh, 40 ml, 60°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:36.00%
Yield based on 100g JA: 7.20 g DP: ND

∑MU extracted: 4.435 mmol
∑DP 1-2 extracted: 0.411 mmol – 9.27%
∑DP 3-4 extracted: 0.657 mmol – 14.81%
∑DP 3-6 extracted (estimated): 0.951 mmol – 21.44%
∑DP 1-2 in JA: 3.34%
∑DP 3-4 in JA: 5.33%
∑DP 3-6 in JA (estimated): 7.72%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.9150 | 0.2030 | 0.2030 |
| Glucose | 0.0540 | 0.0120 | 0.0120 |
| Sucrose | 0.8370 | 0.0980 | 0.1960 |
| Kestose | 1.7070 | 0.1350 | 0.4050 |
| Nystose | 1.0510 | 0.0630 | 0.2520 |
| DP5 | 0.5031 | 0.0240 | 0.1200 |
| DP6 | 0.7275 | 0.0290 | 0.1740 |

Density: 0.978 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.6130 ΔE: 37.32

**EXPERIMENT NO: 81**

Description: April 06 harvested, fresh, 40 ml, 20°C, 3 min, WB, 26 mM acidic, Uncooked, whole

Absorbance@520nm: 1.1842 $Y_{DM}$:30.20%
Yield based on 100g JA: 6.04 g DP: 12.92
∑MU extracted: 3.721 mmol
∑DP 1-2 extracted: 0.448 mmol – 12.04%
∑DP 3-4 extracted: 0.292 mmol – 7.85%
∑DP 3-6 extracted (estimated): 0.592 mmol – 15.91%
∑DP 1-2 in JA: 3.64%
∑DP 3-4 in JA: 2.37%
∑DP 3-6 in JA (estimated): 4.81%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.6621 | 0.1470 | 0.1470 |
| Glucose | 0.3210 | 0.0710 | 0.0710 |
| Sucrose | 0.9833 | 0.1150 | 0.2300 |
| Kestose | 0 | 0 | 0 |
| Nystose | 1.2130 | 0.0730 | 0.2920 |
| DP5 | 0.8676 | 0.0420 | 0.2100 |
| DP6 | 0.3803 | 0.0150 | 0.0900 |

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 82**

Description: April 06 harvested, fresh, 40 ml, 20°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: 0.2758
$Y_{DM}$:24.65%
Yield based on 100g JA: 4.93 g
DP: 6.99
∑MU extracted: 3.037 mmol
∑DP 1-2 extracted: 0.277 mmol – 9.12%
∑DP 3-4 extracted: 0.542 mmol – 17.85%
∑DP 3-6 extracted (estimated): 0.677 mmol – 22.29%
∑DP 1-2 in JA: 2.25%
∑DP 3-4 in JA: 4.40%
∑DP 3-6 in JA (estimated): 5.50%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.5900 | 0.1310 | 0.1310 |
| Glucose | 0.0800 | 0.0180 | 0.0180 |
| Sucrose | 0.5490 | 0.0640 | 0.1280 |
| Kestose | 1.3890 | 0.1100 | 0.3300 |
| Nystose | 0.8900 | 0.0530 | 0.2120 |
| DP5 | 0.1953 | 0.0090 | 0.0450 |
| DP6 | 0.3780 | 0.0150 | 0.0900 |

Density: 0.98 g/ml
Viscosity: 1.01 cp
Absorbance@420 nm: 0.3624
ΔE: 11.95

**EXPERIMENT NO: 83**

Description: April 06 harvested, fresh, 40 ml, 20°C, 3 min, UB + WB, 26mM acidic, Uncooked, whole

Absorbance@520nm: 0.2909
$Y_{DM}$:25.20%
Yield based on 100g JA: 5.04 g
DP: 6.77

∑MU extracted: 3.105 mmol
∑DP 1-2 extracted: 0.4186 mmol – 13.48%
∑DP 3-4 extracted: 0.54 mmol – 17.39%
∑DP 3-6 extracted (estimated): 0.677 mmol – 21.80%
∑DP 1-2 in JA: 3.40%
∑DP 3-4 in JA: 4.38%
∑DP 3-6 in JA (estimated): 5.50%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.4234 | 0.3160 | 0.3160 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0.4390 | 0.0513 | 0.1026 |
| Kestose | 0.9105 | 0.0720 | 0.2160 |
| Nystose | 1.3537 | 0.0810 | 0.3240 |
| DP5 | 0.2673 | 0.0130 | 0.0650 |
| DP6 | 0.2910 | 0.0120 | 0.0720 |

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 84**

Description: April 06 harvested, 5 day stored, 40 ml, 20°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:42.44%
Yield based on 100g JA: 8.49 g
DP: ND
∑MU extracted: 5.229 mmol
∑DP 1-2 extracted: 0.464 mmol – 8.87%
∑DP 3-4 extracted: 0.563 mmol – 10.77%
∑DP 3-6 extracted (estimated): 0.975 mmol – 18.65%
∑DP 1-2 in JA: 3.77%
∑DP 3-4 in JA: 4.57%
∑DP 3-6 in JA (estimated): 7.91%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.9606 | 0.2130 | 0.2130 |
| Glucose | 0.1575 | 0.0350 | 0.0350 |
| Sucrose | 0.9225 | 0.1080 | 0.2160 |
| Kestose | 1.2244 | 0.0970 | 0.2910 |
| Nystose | 1.1309 | 0.0680 | 0.2720 |
| DP5 | 0.7950 | 0.0380 | 0.1900 |
| DP6 | 0.9220 | 0.0370 | 0.2220 |

Density: 0.976 g/ml Viscosity: 1.00 cp
Absorbance@420 nm: 1.4146 ΔE: 37.42

**EXPERIMENT NO: 85**

Description: April 06 harvested, 10 day stored, 40 ml, 20°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:58.55%
Yield based on 100g JA: 11.71 g DP: ND
∑MU extracted: 7.213 mmol
∑DP 1-2 extracted: 0.488 mmol – 6.77%
∑DP 3-4 extracted: 0.753 mmol – 10.44%
∑DP 3-6 extracted (estimated): 0.584 mmol – 8.10%
∑DP 1-2 in JA: 3.96%
∑DP 3-4 in JA: 6.11%
∑DP 3-6 in JA (estimated): 4.74%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.4710 | 0.1050 | 0.1050 |
| Glucose | 0.0860 | 0.0190 | 0.0190 |
| Sucrose | 1.5560 | 0.1820 | 0.3640 |
| Kestose | 1.9530 | 0.1550 | 0.4650 |
| Nystose | 1.1930 | 0.0720 | 0.2880 |
| DP5 | 1.1990 | 0.0580 | 0.2900 |
| DP6 | 1.2030 | 0.0490 | 0.2940 |

Density: 0.978 g/ml Viscosity: 1.00 cp
Absorbance@420 nm: 1.4239 ΔE: 37.73

**EXPERIMENT NO: 86**

Description: April 06 harvested, 15 day stored, 40 ml, 20°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:61.03%
Yield based on 100g JA: 12.21 g DP: ND

∑MU extracted: 7.520 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 87**

Description: April 06 harvested,20 day stored, 40 ml, 20°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:63.17%
Yield based on 100g JA: 12.63 g
DP: ND
∑MU extracted: 7.780 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

**EXPERIMENT NO: 88**

Description: April 06 harvested, 5 day stored, 40 ml, 40°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:42.57%
Yield based on 100g JA: 4.51 g
DP: ND
∑MU extracted: 5.245 mmol
∑DP 1-2 extracted: 0.2974 mmol – 5.67%
∑DP 3-4 extracted: 0.2558 mmol – 4.88%
∑DP 3-6 extracted (estimated): 0.5266 mmol – 10.04%
∑DP 1-2 in JA: 2.41%
∑DP 3-4 in JA: 2.08%
∑DP 3-6 in JA (estimated): 4.27%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.2713 | 0.1487 | 0.2974 |
| Kestose | 0.2955 | 0.0234 | 0.0702 |
| Nystose | 0.7729 | 0.0464 | 0.1856 |
| DP5 | 0.9018 | 0.0436 | 0.2180 |
| DP6 | 0.2167 | 0.0088 | 0.0528 |

Density: 0.979 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.6125 ΔE: 37.97

**EXPERIMENT NO: 89**

Description: April 06 harvested, 10 day stored, 40 ml, 40°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:51.73%
Yield based on 100g JA: 6.35 g DP: ND
∑MU extracted: 6.373 mmol
∑DP 1-2 extracted: 0.6424 mmol – 10.08%
∑DP 3-4 extracted: 0.3486 mmol – 5.47%
∑DP 3-6 extracted (estimated): 0.6891 mmol – 10.81%
∑DP 1-2 in JA: 5.21%
∑DP 3-4 in JA: 2.83%
∑DP 3-6 in JA (estimated): 5.59%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.9575 | 0.4350 | 0.4350 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0.8865 | 0.1037 | 0.2074 |
| Kestose | 0.1845 | 0.0146 | 0.0438 |
| Nystose | 1.2679 | 0.0762 | 0.3048 |
| DP5 | 0.8510 | 0.0411 | 0.2055 |
| DP6 | 0.5560 | 0.0225 | 0.1350 |

Density: 0.984 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.6312 ΔE: 38.18

## EXPERIMENT NO: 90

Description: April 06 harvested, 15 day stored, 40 ml, 40°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$: 62.20%
Yield based on 100g JA: 6.44 g
DP: ND
∑MU extracted: 7.660 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND
Density: ND
Viscosity: ND
Absorbance@420 nm: ND
ΔE: ND

## EXPERIMENT NO: 91

Description: April 06 harvested, 20 day stored, 40 ml, 40°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:62.47%
Yield based on 100g JA: 6.49 g
DP: ND
∑MU extracted: 7.700 mmol
∑DP 1-2 extracted: 0.2566 mmol – 3.33%
∑DP 3-4 extracted: 0.8079 mmol – 10.49%
∑DP 3-6 extracted (estimated): 0.9564 mmol – 12.42%
∑DP 1-2 in JA: 2.08%
∑DP 3-4 in JA: 6.56%
∑DP 3-6 in JA (estimated): 7.76%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.0970 | 0.1283 | 0.2566 |
| Kestose | 2.4100 | 0.1913 | 0.5739 |
| Nystose | 0.9740 | 0.0585 | 0.2340 |
| DP5 | 0.1935 | 0.0093 | 0.0465 |
| DP6 | 0.4215 | 0.0170 | 0.1020 |

Density: 0.985 g/ml
Viscosity: 1.03 cp
Absorbance@420 nm: 2.0439
ΔE: 40.25

**EXPERIMENT NO: 92**

Description: April 06 harvested, 5 day stored, 40 ml, 60°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:47.03%
Yield based on 100g JA: 9.41 g
DP: ND
∑MU extracted: 5.794 mmol
∑DP 1-2 extracted: 0.7892 mmol – 13.62%
∑DP 3-4 extracted: 0.2 mmol – 3.45%
∑DP 3-6 extracted (estimated): 0.985 mmol – 17.00%
∑DP 1-2 in JA: 6.41%
∑DP 3-4 in JA: 1.62%
∑DP 3-6 in JA (estimated): 8.00%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.3555 | 0.0790 | 0.0790 |
| Glucose | 0.7020 | 0.1560 | 0.1560 |
| Sucrose | 2.3693 | 0.2771 | 0.5542 |
| Kestose | 0 | 0 | 0 |
| Nystose | 0.8341 | 0.0500 | 0.2000 |
| DP5 | 1.9085 | 0.0922 | 0.4610 |
| DP6 | 1.3357 | 0.0540 | 0.3240 |

Density: 0.981 g/ml
Viscosity: 1.01 cp
Absorbance@420 nm: 1.6234
ΔE: 38.06

**EXPERIMENT NO: 93**

Description: April 06 harvested, 10 day stored, 40 ml, 60°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:54.32%
Yield based on 100g JA: 10.86 g
DP: ND
∑MU extracted: 6.692mmol
∑DP 1-2 extracted: 0 mmol – 0%
∑DP 3-4 extracted: 0.3736 mmol – 5.58%
∑DP 3-6 extracted (estimated): 1.1561 mmol – 17.27%

∑DP 1-2 in JA: 0%
∑DP 3-4 in JA: 3.03%
∑DP 3-6 in JA (estimated): 9.38%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 0 | 0 | 0 |
| Nystose | 1.5554 | 0.0934 | 0.3736 |
| DP5 | 1.5250 | 0.0737 | 0.3685 |
| DP6 | 1.7070 | 0.0690 | 0.4140 |

Density: 0.986 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 1.6749 ΔE: 38.27

**EXPERIMENT NO: 94**

Description: April 06 harvested, 15 day stored, 40 ml, 60°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:63.47%
Yield based on 100g JA: 12.69 g DP: ND
∑MU extracted: 7.820 mmol
∑DP 1-2 extracted: ND
∑DP 3-4 extracted: ND
∑DP 3-6 extracted (estimated): ND

Density: ND Viscosity: ND
Absorbance@420 nm: ND ΔE: ND

**EXPERIMENT NO: 95**

Description: April 06 harvested, 20 day stored, 40 ml, 60°C, 3.5 min, UB, NA, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:64.87%
Yield based on 100g JA: 12.97 g DP: ND
∑MU extracted: 7.992 mmol
∑DP 1-2 extracted: 1.0736 mmol – 13.43%
∑DP 3-4 extracted: 1.1938 mmol – 14.94%
∑DP 3-6 extracted (estimated): 1.8008 mmol – 22.53%
∑DP 1-2 in JA: 8.71%
∑DP 3-4 in JA: 9.69%
∑DP 3-6 in JA (estimated): 14.62%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.7995 | 0.4000 | 0.4000 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 2.8800 | 0.3368 | 0.6736 |
| Kestose | 3.0970 | 0.2458 | 0.7374 |
| Nystose | 1.9000 | 0.1141 | 0.4564 |
| DP5 | 1.1970 | 0.0578 | 0.2890 |
| DP6 | 1.3120 | 0.0530 | 0.3180 |

Density: 0.988 g/ml Viscosity: 1.25 cp
Absorbance@420 nm: 2.1101 ΔE: 40.37

**EXPERIMENT NO: 96**

Description: April 06 harvested, fresh, 40 ml, 40°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:58.37%
Yield based on 100g JA: 11.67 g DP: ND
∑MU extracted: 7.191 mmol
∑DP 1-2 extracted: 0.656 mmol – 9.12%
∑DP 3-4 extracted: 0.821 mmol – 11.42%
∑DP 3-6 extracted (estimated): 1.48 mmol – 20.58%
∑DP 1-2 in JA: 5.32%
∑DP 3-4 in JA: 6.66%
∑DP 3-6 in JA (estimated): 12.01%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2330 | 0.0520 | 0.0520 |
| Glucose | 0.1510 | 0.0340 | 0.0340 |
| Sucrose | 2.1060 | 0.2850 | 0.5700 |
| Kestose | 2.1540 | 0.1670 | 0.5010 |
| Nystose | 1.3280 | 0.0800 | 0.3200 |
| DP5 | 1.3810 | 0.0670 | 0.3350 |
| DP6 | 1.3270 | 0.0540 | 0.3240 |

Density: 0.981 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 0.3728 ΔE: 12.09

**EXPERIMENT NO: 97**

Description: April 06 harvested, fresh, 40 ml, 60°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:64.24%
Yield based on 100g JA: 14.85 g DP: ND
∑MU extracted: 9.146 mmol
∑DP 1-2 extracted: 0.706 mmol – 7.72%
∑DP 3-4 extracted: 1.195 mmol – 13.07%
∑DP 3-6 extracted (estimated): 2.043 mmol – 22.34%
∑DP 1-2 in JA: 5.73%
∑DP 3-4 in JA: 9.70%
∑DP 3-6 in JA (estimated): 16.58%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2520 | 0.0560 | 0.0560 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 2.9910 | 0.3250 | 0.6500 |
| Kestose | 3.4430 | 0.2730 | 0.8190 |
| Nystose | 1.5620 | 0.0940 | 0.3760 |
| DP5 | 2.4417 | 0.1180 | 0.5900 |
| DP6 | 1.0790 | 0.0430 | 0.2580 |

Density: 0.983 g/ml Viscosity: 1.12 cp
Absorbance@420 nm: 0.3799 ΔE: 12.73

**EXPERIMENT NO: 98**

Description: April 06 harvested, 5 day stored, 40 ml, 20°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND
Yield based on 100g JA: 5.40 g
$Y_{DM}$:27.01%
DP: ND
∑MU extracted: 3.328 mmol
∑DP 1-2 extracted: 0.275 mmol – 8.26%
∑DP 3-4 extracted: 0.192 mmol – 5.77%
∑DP 3-6 extracted (estimated): 0.541 mmol – 16.26%
∑DP 1-2 in JA: 2.23%
∑DP 3-4 in JA: 1.56%
∑DP 3-6 in JA (estimated): 4.39%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1661 | 0.0370 | 0.0370 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.0190 | 0.1190 | 0.2380 |
| Kestose | 0 | 0 | 0 |
| Nystose | 0.8063 | 0.0480 | 0.1920 |
| DP5 | 0.7190 | 0.0350 | 0.1750 |
| DP6 | 0.7130 | 0.0290 | 0.1740 |

Density: 0.987 g/ml
Absorbance@420 nm: 0.5148
Viscosity: 1.01 cp
ΔE: 12.62

**EXPERIMENT NO: 99**

Description: April 06 harvested, 10 day stored, 40 ml, 20°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND
Yield based on 100g JA: 5.63 g
$Y_{DM}$:28.16%
DP: ND
∑MU extracted: 3.470 mmol
∑DP 1-2 extracted: 0.059 mmol – 1.70%
∑DP 3-4 extracted: 0.216 mmol – 6.22%
∑DP 3-6 extracted (estimated): 0.576 mmol – 16.60%
∑DP 1-2 in JA: 0.48%

∑DP 3-4 in JA: 1.75%
∑DP 3-6 in JA (estimated): 4.68%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.2668 | 0.0590 | 0.0590 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 0 | 0 | 0 |
| Nystose | 0.8935 | 0.0540 | 0.2160 |
| DP5 | 0.7490 | 0.0360 | 0.1800 |
| DP6 | 0.7510 | 0.0300 | 0.1800 |

Density: 0.989 g/ml Viscosity: 1.01 cp
Absorbance@420 nm: 0.6529 ΔE: 13.34

**EXPERIMENT NO: 100**

Description: April 06 harvested, 15 day stored, 40 ml, 20°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:34.61%
Yield based on 100g JA: 8.92 g DP: ND
∑MU extracted: 4.264 mmol
∑DP 1-2 extracted: 0.256 mmol – 6.00%
∑DP 3-4 extracted: 0.805 mmol – 18.88%
∑DP 3-6 extracted (estimated): 1.196 mmol – 28.00%
∑DP 1-2 in JA: 2.08%
∑DP 3-4 in JA: 6.53%
∑DP 3-6 in JA (estimated): 9.71%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.0970 | 0.1280 | 0.2560 |
| Kestose | 2.4100 | 0.1910 | 0.5730 |
| Nystose | 0.9740 | 0.0580 | 0.2320 |
| DP5 | 0.8400 | 0.0410 | 0.2050 |
| DP6 | 0.7600 | 0.0310 | 0.1860 |

Density: 0.993 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 0.7360 ΔE: 13.65

**EXPERIMENT NO: 101**

Description: April 06 harvested, 20 day stored, 40 ml, 20°C, 3 min, UB, 26mM acidic, Uncooked

Absorbance@520nm: ND
$Y_{DM}$:35.62%
Yield based on 100g JA: 9.10 g
DP: ND
∑MU extracted: 4.388 mmol
∑DP 1-2 extracted: 0.2496 mmol – 5.69%
∑DP 3-4 extracted: 0.786 mmol – 17.91%
∑DP 3-6 extracted (estimated): 1.319 mmol – 30.00%
∑DP 1-2 in JA: 2.03%
∑DP 3-4 in JA: 6.38%
∑DP 3-6 in JA (estimated): 10.71%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.0496 | 0.0110 | 0.0110 |
| Glucose | 0.0297 | 0.0066 | 0.0066 |
| Sucrose | 0.9909 | 0.1160 | 0.2320 |
| Kestose | 2.0445 | 0.1620 | 0.4860 |
| Nystose | 1.2454 | 0.0750 | 0.3000 |
| DP5 | 1.8873 | 0.0910 | 0.4550 |
| DP6 | 0.3195 | 0.0130 | 0.0780 |

Density: 0.995 g/ml
Viscosity: 1.02 cp
Absorbance@420 nm: 0.7414
ΔE: 14.26

**EXPERIMENT NO: 102**

Description: April 06 harvested, 5 day stored, 40 ml, 40°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$:60.10%
Yield based on 100g JA: 4.02 g
DP: ND
∑MU extracted: 7.404 mmol
∑DP 1-2 extracted: 0.071 mmol – 0.96%
∑DP 3-4 extracted: 0.1476 mmol – 2.00%
∑DP 3-6 extracted (estimated): 0.4309 mmol – 5.82%
∑DP 1-2 in JA: 0.58%

∑DP 3-4 in JA: 1.20%
∑DP 3-6 in JA (estimated): 3.50%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1435 | 0.0320 | 0.0320 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0.1667 | 0.0195 | 0.0390 |
| Kestose | 0 | 0 | 0 |
| Nystose | 0.6142 | 0.0369 | 0.1476 |
| DP5 | 0.5580 | 0.0269 | 0.1345 |
| DP6 | 0.6130 | 0.0248 | 0.1488 |

Density: 0.989 g/ml
Viscosity: 1.01 cp
Absorbance@420 nm: 0.5644
ΔE: 12.89

**EXPERIMENT NO: 103**

Description: April 06 harvested, 10 day stored, 40 ml, 40°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND
$Y_{DM}$: 62.45%
Yield based on 100g JA: 8.49 g
DP: ND
∑MU extracted: 7.690 mmol
∑DP 1-2 extracted: 0.343 mmol – 4.46%
∑DP 3-4 extracted: 0.7238 mmol – 9.41%
∑DP 3-6 extracted (estimated): 1.1217 mmol – 14.59%
∑DP 1-2 in JA: 2.78%
∑DP 3-4 in JA: 5.88%
∑DP 3-6 in JA (estimated): 9.10%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1530 | 0.0340 | 0.0340 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.3210 | 0.1545 | 0.3090 |
| Kestose | 1.9375 | 0.1538 | 0.4614 |
| Nystose | 1.0930 | 0.0656 | 0.2624 |
| DP5 | 0.8790 | 0.0425 | 0.2125 |
| DP6 | 0.7640 | 0.0309 | 0.1854 |

Density: 0.993 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 0.6635 ΔE: 14.10

**EXPERIMENT NO: 104**

Description: April 06 harvested, 15 day stored, 40 ml, 40°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$: 65.90%
Yield based on 100g JA: 9.18 g DP: ND
∑MU extracted: 8.119 mmol
∑DP 1-2 extracted: 0.48 mmol – 5.91%
∑DP 3-4 extracted: 0.5436 mmol – 6.70%
∑DP 3-6 extracted (estimated): 0.9881 mmol – 12.17%
∑DP 1-2 in JA: 3.90%
∑DP 3-4 in JA: 4.41%
∑DP 3-6 in JA (estimated): 8.02%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0.1454 | 0.0320 | 0.0320 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.9153 | 0.2240 | 0.4480 |
| Kestose | 1.2553 | 0.0996 | 0.2988 |
| Nystose | 1.0198 | 0.0612 | 0.2448 |
| DP5 | 0.9960 | 0.0481 | 0.2405 |
| DP6 | 0.9850 | 0.0340 | 0.2040 |

Density: 0.995 g/ml Viscosity: 1.02 cp
Absorbance@420 nm: 0.7982 ΔE: 14.77

**EXPERIMENT NO: 105**

Description: April 06 harvested, 20 day stored, 40 ml, 40°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:68.10%
Yield based on 100g JA: 10.61 g DP: ND

∑MU extracted: 8.390 mmol
∑DP 1-2 extracted: 0.3204 mmol – 3.82%
∑DP 3-4 extracted: 0.8845 mmol – 10.54%
∑DP 3-6 extracted (estimated): 1.3275 mmol – 15.82%
∑DP 1-2 in JA: 2.60%
∑DP 3-4 in JA: 7.18%
∑DP 3-6 in JA (estimated): 10.78%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.3694 | 0.1602 | 0.3204 |
| Kestose | 2.1765 | 0.1727 | 0.5181 |
| Nystose | 1.5244 | 0.0916 | 0.3664 |
| DP5 | 0.8766 | 0.0424 | 0.2120 |
| DP6 | 0.9525 | 0.0385 | 0.2310 |

Density: 0.996 g/ml Viscosity: 1.04 cp
Absorbance@420 nm: 0.8202 ΔE: 15.16

**EXPERIMENT NO: 106**

Description: April 06 harvested, 5 day stored, 40 ml, 60°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:79.66%
Yield based on 100g JA: 5.93 g DP: ND
∑MU extracted: 9.810 mmol
∑DP 1-2 extracted: 0.1157 mmol – 1.18%
∑DP 3-4 extracted: 0.266 mmol – 2.71%
∑DP 3-6 extracted (estimated): 0.6889 mmol – 7.02%
∑DP 1-2 in JA: 0.94%
∑DP 3-4 in JA: 2.16%
∑DP 3-6 in JA (estimated): 5.59%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0.4084 | 0.0910 | 0.0910 |
| Sucrose | 0.1055 | 0.0123 | 0.0247 |
| Kestose | 0 | 0 | 0 |
| Nystose | 1.1073 | 0.0665 | 0.2660 |
| DP5 | 0.8710 | 0.0421 | 0.2105 |
| DP6 | 0.8760 | 0.0354 | 0.2124 |

Density: 0.986 g/ml Viscosity: 1.12 cp
Absorbance@420 nm: 0.5714 ΔE: 13.44

**EXPERIMENT NO: 107**

Description: April 06 harvested, 10 day stored, 40 ml, 60°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:79.73%
Yield based on 100g JA: 11.28 g DP: ND
∑MU extracted: 9.820 mmol
∑DP 1-2 extracted: 0 mmol – 0%
∑DP 3-4 extracted: 1.3411 mmol – 19.30%
∑DP 3-6 extracted (estimated): 1.6857 mmol – 24.25%
∑DP 1-2 in JA: 0%
∑DP 3-4 in JA: 10.89%
∑DP 3-6 in JA (estimated): 13.68%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 0 | 0 | 0 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0 | 0 | 0 |
| Kestose | 3.3935 | 0.2693 | 0.8079 |
| Nystose | 2.2190 | 0.1333 | 0.5332 |
| DP5 | 0.7780 | 0.0376 | 0.1880 |
| DP6 | 3.5145 | 0.1420 | 0.8519 |

Density: 0.994 g/ml Viscosity: 1.22 cp
Absorbance@420 nm: 0.7319 ΔE: 14.63

**EXPERIMENT NO: 108**

Description: April 06 harvested, 20 day stored, 40 ml, 60°C, 3 min, UB, 26mM acidic, Uncooked, whole

Absorbance@520nm: ND $Y_{DM}$:57.39%
Yield based on 100g JA: 11.48 g DP: ND
∑MU extracted: 7.070 mmol

∑DP 1-2 extracted: 0.5564 mmol – 7.87%
∑DP 3-4 extracted: 0.9728 mmol – 13.76%
∑DP 3-6 extracted (estimated): 1.5841 mmol – 22.41%
∑DP 1-2 in JA: 4.52%
∑DP 3-4 in JA: 7.90%
∑DP 3-6 in JA (estimated): 12.86%

| | Concentration (mg/ml) | Amount (mmol) | MU (mmol) |
|---|---|---|---|
| Fructose | 1.4825 | 0.3290 | 0.3290 |
| Glucose | 0 | 0 | 0 |
| Sucrose | 0.9720 | 0.1137 | 0.2274 |
| Kestose | 2.4045 | 0.1908 | 0.5724 |
| Nystose | 1.6750 | 0.1001 | 0.4004 |
| DP5 | 1.2200 | 0.0589 | 0.2945 |
| DP6 | 1.3080 | 0.0528 | 0.3168 |

Density: 1.0112 g/ml
Viscosity: 1.35 cp
Absorbance@420 nm: 0.8891
ΔE: 15.88

# APPENDIX D

## SAMPLE CALCULATIONS

### 1. Calculation of analytical yield:

If the dry matter content of JA is 20%, and 10 g of JA was used;

$$\text{Dry matter in JA} = 10\text{g} \,.\, (0.20) = 2\text{ g}$$

Thus by using the formula given in Chapter 2, the yield for Experiment 38;

$$\text{Yield} = (1.6528 / 2).\ 100 = 82.64\%$$

### 2. Calculation of DP:

By using the formulas given in Chapter 2, for Experiment 38;

$$\text{RE (mg G)} = (0.919 / 0.143) \,.\, 40 = 257\text{ mg G}$$

$$\text{DP} = \{[(1.6528 \,.\, 10^3 / 257) \,.\, 180] - 18\} \,.\, (1 / 162) = 7.03$$

### 3. Calculation of ∑MU extracted:

Assuming $DP_{av}$ in JA is 60;

$$\sum\text{MU in JA} = [2000\text{ mg} / (59 \,.\, 162 + 180)] \,.\, 60 = 12.32\text{ mmol}$$

Thus for Experiment 38;

$$\sum MU \text{ extracted} = [\text{Yield} \cdot (12.32)] / 100 = 10.181 \text{ mmol}$$

## 4. Calculation of amount of sugars:

$$\text{Amount of sugar (mmol)} = [(\text{Concentration of sugar}) / (MW_{sugar})] \cdot 40 \text{ (ml)}$$

in where $MW_{fructose} = 180$, $MW_{glucose} = 180$, $MW_{sucrose} = 342$, $MW_{kestose} = 504$, $MW_{nystose} = 666$, $MW_{DP5} = 828$, $MW_{DP6} = 990$.

For Experiment 38;

Amount of Fructose = [(0.3631 / 180) x 40] = 0.0807 mmol

Amount of Glucose = [(0.0814 / 180) x 40] = 0.0181 mmol

Amount of Sucrose = [(3.263 / 342) x 40] = 0.3816 mmol

Amount of Kestose = [(0.9545 / 504) x 40] = 0.0757 mmol

Amount of Nystose = [(1.3363 / 666) x 40] = 0.0803 mmol

Amount of DP5 = [(0.4809 / 828) x 40] = 0.0232 mmol

Amount of DP6 = [(1.3722 / 990) x 40] = 0.0554 mmol

## 5. Calculation of MU in sugars:

$$\text{MU in sugar (mmol)} = \text{Amount of sugar (mmol)} \cdot \text{DP of sugar}$$

For Experiment 38;

MU in fructose = (0.0807) . 1 = 0.0807 mmol

MU in glucose = (0.0181) . 1 = 0.0181 mmol

MU in sucrose = (0.3816) . 2 = 0.7632 mmol

MU in kestose = (0.0757) . 3 = 0.2271 mmol

MU in nystose = (0.0803) . 4 = 0.3212 mmol

MU in DP5 = (0.0232) . 5 = 0.116 mmol

MU in DP6 = (0.0554) . 6 = 0.3324 mmol

### 6. Calculation of % of ∑DP 1-2 extracted and in JA sample:

∑DP 1-2 extracted (%) = [(∑MU in glucose, fructose and sucrose) / (∑MU extracted)] . 100

For Experiment 38;

∑DP 1-2 extracted (%) = [(0.0807 + 0.0181 + 0.7632) / (10.181)] . 100 = 8.47%

∑DP 1-2 in JA sample (%) = [(∑MU in glucose, fructose and sucrose) / (∑MU in JA sample)] . 100

For Experiment 38;

∑DP 1-2 extracted (%) = [(0.0807 + 0.0181 + 0.7632) / (12.32)] . 100 = 7%

### 7. Calculation of % of ∑DP 3-4 extracted and in JA sample:

∑DP 3-4 extracted (%) = [(∑MU in kestose and nystose) / (∑MU extracted)] . 100

For Experiment 38;

∑DP 3-4 extracted (%) = [(0.2271 + 0.3212) / (10.181)] . 100 = 5.39%

∑DP 3-4 in JA sample (%) = [(∑MU in kestose and nystose) / (∑MU in JA sample)] . 100

For Experiment 38;

∑DP 3-4 extracted (%) = [(0.2271 + 0.3212) / (12.32)] . 100 = 4.45%

## 8. Calculation of % of ∑DP 3-6 extracted and in JA sample:

$$\sum DP\ 3\text{-}6\ extracted\ (\%) = [(\sum MU\ in\ kestose,\ nystose,\ DP5\ and\ DP6) / (\sum MU\ extracted)] \cdot 100$$

For Experiment 38;

$$\sum DP\ 3\text{-}6\ extracted\ (\%) = [(0.2271 + 0.3212 + 0.116 + 0.3324) / (10.181)] \cdot 100$$

$$\sum DP\ 3\text{-}6\ extracted\ (\%) = 9.79\%$$

$$\sum DP\ 3\text{-}6\ in\ JA\ sample\ (\%) = [(\sum MU\ in\ kestose,\ nystose,\ DP5\ and\ DP6) / (\sum MU\ in\ JA\ sample)] \cdot 100$$

For Experiment 38;

$$\sum DP\ 3\text{-}6\ extracted\ (\%) = [(0.2271 + 0.3212 + 0.116 + 0\ 3324) / (12.32)] \cdot 100$$

$$\sum DP\ 3\text{-}6\ extracted\ (\%) = 8.09\%$$

## 9. Application of Student's t distribution test:

For example to determine whether the harvest date or storage time cause any significant change in densities of the syrups under non-acidic conditions:

The measured data were as follows:

| | February (A) | March (B) | April (C) | |
|---|---|---|---|---|
| Fresh | 0.962 | 0.959 | 0.958 | H |
| 5 day-stored | 0.966 | 0.965 | 0.964 | G |
| 10 day-stored | 0.974 | 0.969 | 0.967 | F |
| 15 day-stored | 0.979 | 0.974 | 0.973 | E |
| 20 day-stored | 0.981 | 0.977 | 0.974 | D |
| SUM | 4.862 | 4.844 | 4.836 | |
| AVERAGE | 0.972 | 0.969 | 0.967 | |

According to the method calculated values for each case as follows:

$Sum_A = (0.962 + 0.966 + 0.974 + 0.979 + 0\ 981) = 4.862$

$Average_A = 4.862 / 5 = 0.972$

$Variance_A^2 = [(0.962 – 0.9724)^2 + (0.966 – 0.9724)^2 + (0.974 – 0.9724)^2 + (0.979 – 0.9724)^2 + (0\ 981 – 0.9724)^2] / (4 -1) = 1.25$

Thus according to the definition

$S_{AB}^2 = [(n_A – 1)\ Variance_A^2 + (n_B – 1)\ Variance_B^2 ] / (5 + 5 – 2) = 1.25$

Assuming the hypothesis that no significant change was observed in density measurements in extracts of February and March-harvested JAs, that is $\eta_A = \eta_B$;

$t_o = (Average_A – Average_B) / [S_{AB} * (1/5 + 1/5)^{1/2}] = 0.1286$ with 8 degrees of freedom.

Since the probability of the assumption was found as 67% by using the table of probability with probability level of 95% [146], the assumption was true.

As another example; for another assumption that no significant change was observed in density measurements in extracts of fresh and 5 day-stored JAs, that is $\eta_H = \eta_G$;

$t_o = (Average_H – Average_G) / [S_{HG} * (1/5 + 1/5)^{1/2}] = 0.00106$ with 4 degrees of freedom.

Since the probability of the assumption was found as 74% by using the table of probability [146], the assumption was true.

Thus by using the same method for all other cases, it was concluded that, no significant change in density of the syrups due to harvest date and storage time was observed.

Since no significant change was observed the average of all values measured were taken as the syrup density as 0.969 g/ml.

# APPENDIX E

## CHROMATOGRAMS

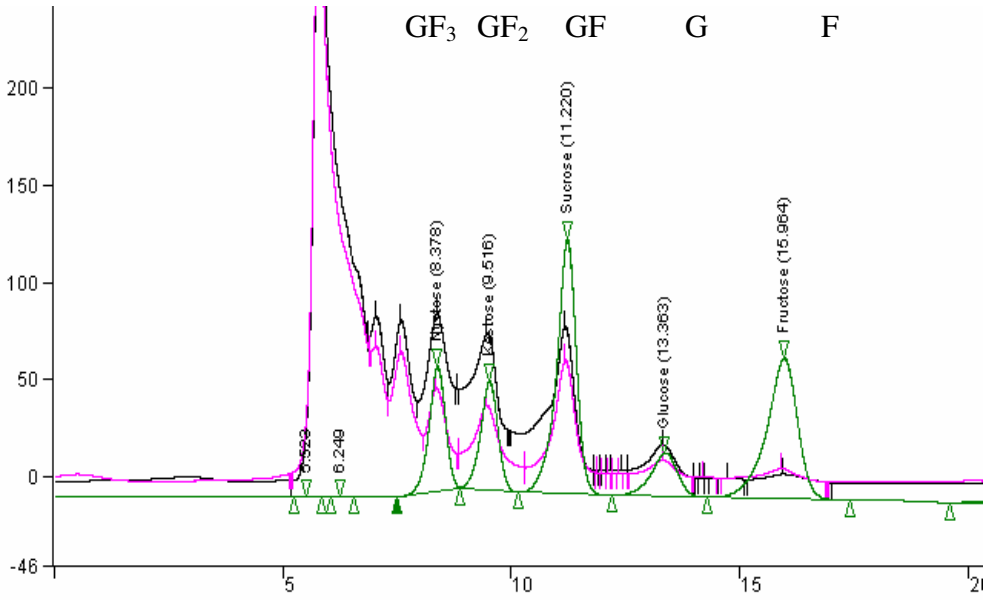


Figure E1. HPLC chromatogram of extracts of February 2006-harvested fresh jerusalem artichoke tubers. Black for acidic, pink for non-acidic extractions, green represents the standart. G: Glucose, F: fructose.

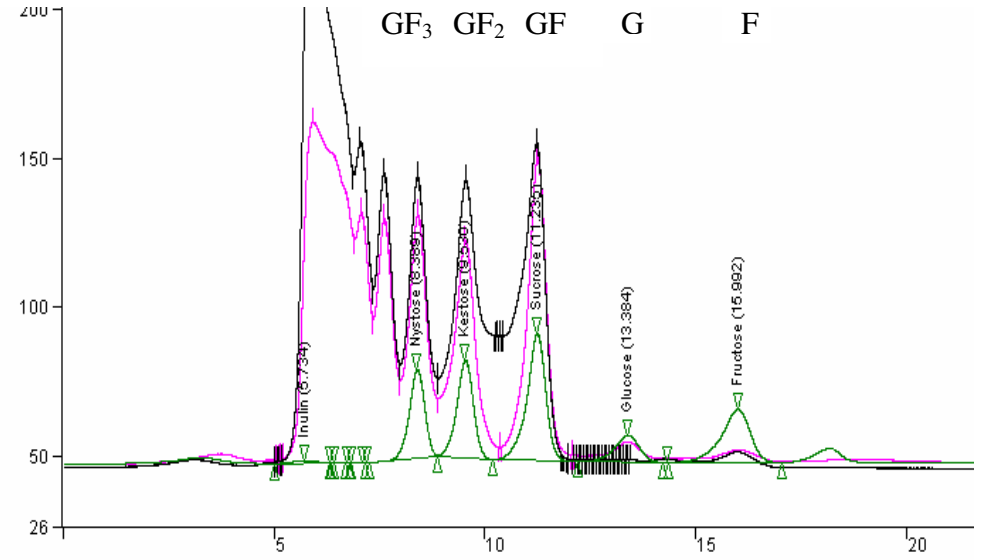


Figure E2. HPLC chromatogram of extracts of February 2006-harvested 20 day-stored jerusalem artichoke tubers. Black for acidic, pink for non-acidic extractions, green represents the standart.

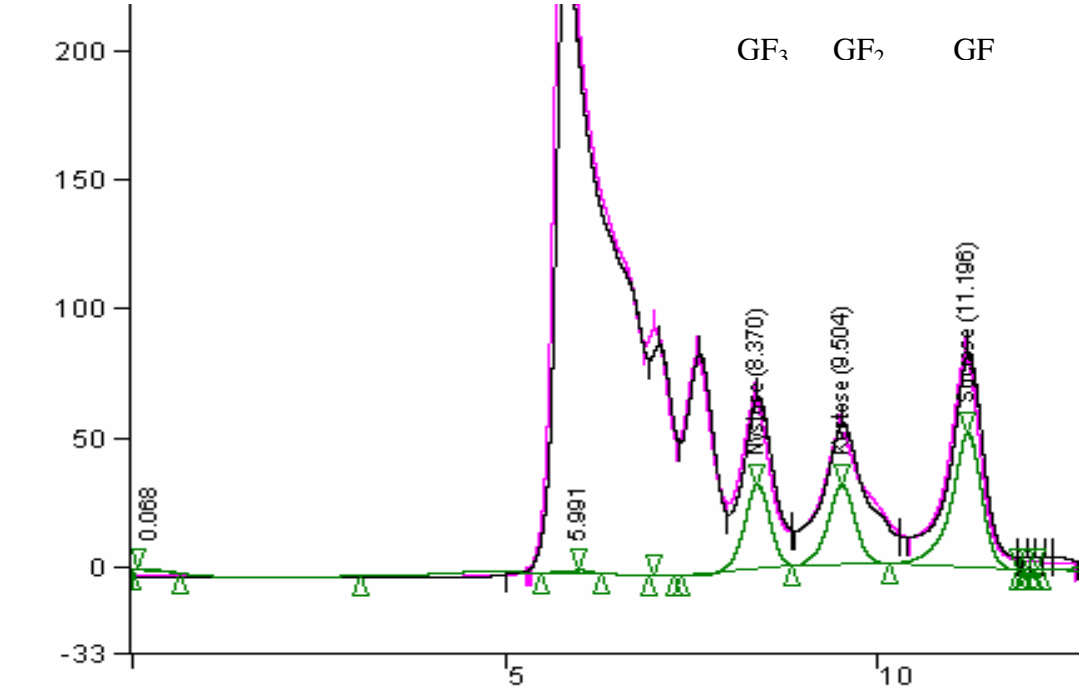


Figure E3. HPLC chromatogram of extracts of April 2006-harvested fresh jerusalem artichoke tubers. Black for acidic, pink for non-acidic extractions, green represents the standart.

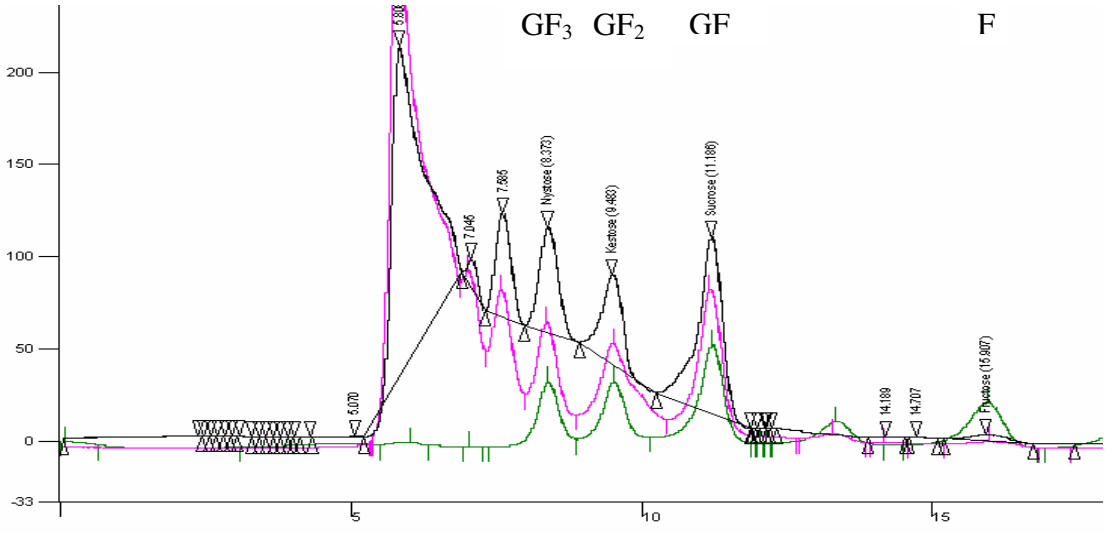


Figure E4. HPLC chromatogram of February 2006-harvested fresh jerusalem artichoke tubers under acidic conditions. Black for uncooked, pink for 1-min-microwaved tubers' extractions, green represents the standart.

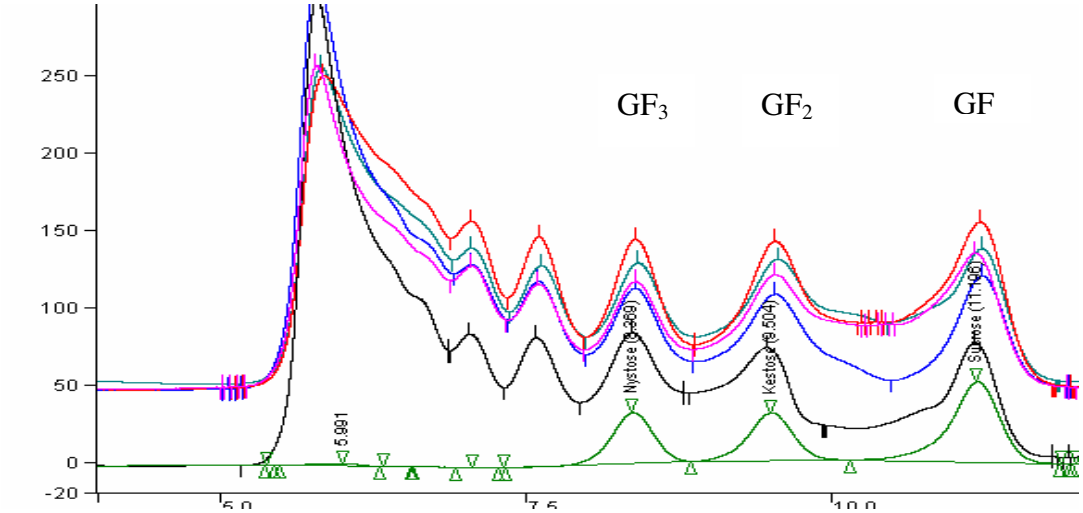


Figure E5. HPLC chromatogram of February 2006-harvested jerusalem artichoke tubers under acidic conditions. Black for fresh, pink for 5 day-stored, blue for 10 day stored, turquoise for 15 day-stored, orange for 20 day-stored, and green represents the standart.

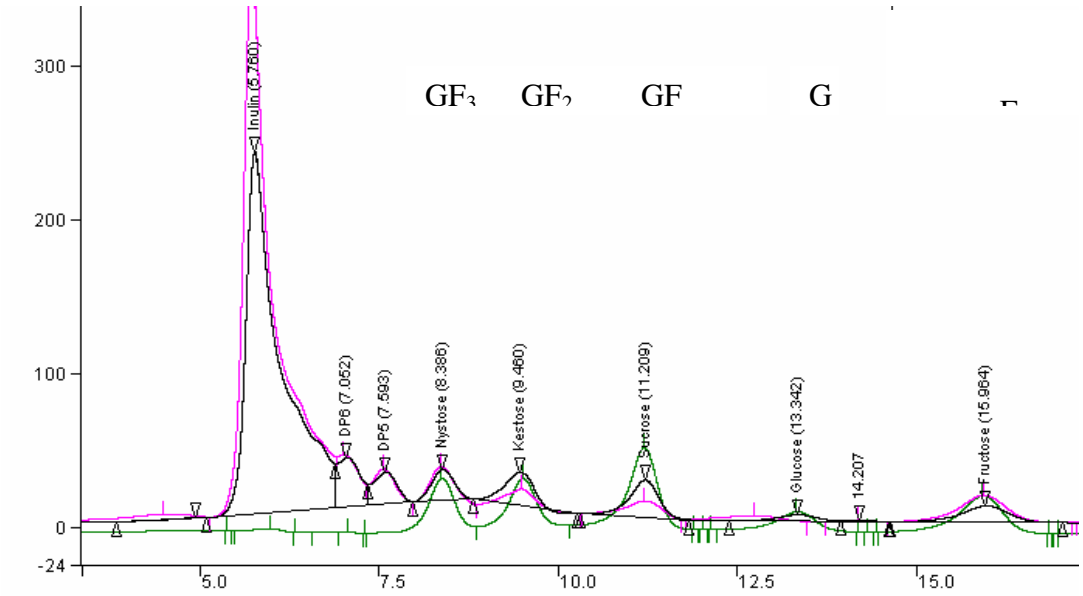


Figure E6. HPLC chromatogram of April 2006-harvested fresh jerusalem artichoke tubers under non-acidic conditions. Black for water-bath extraction, pink for ultrasonic bath extraction, green represents the standart.

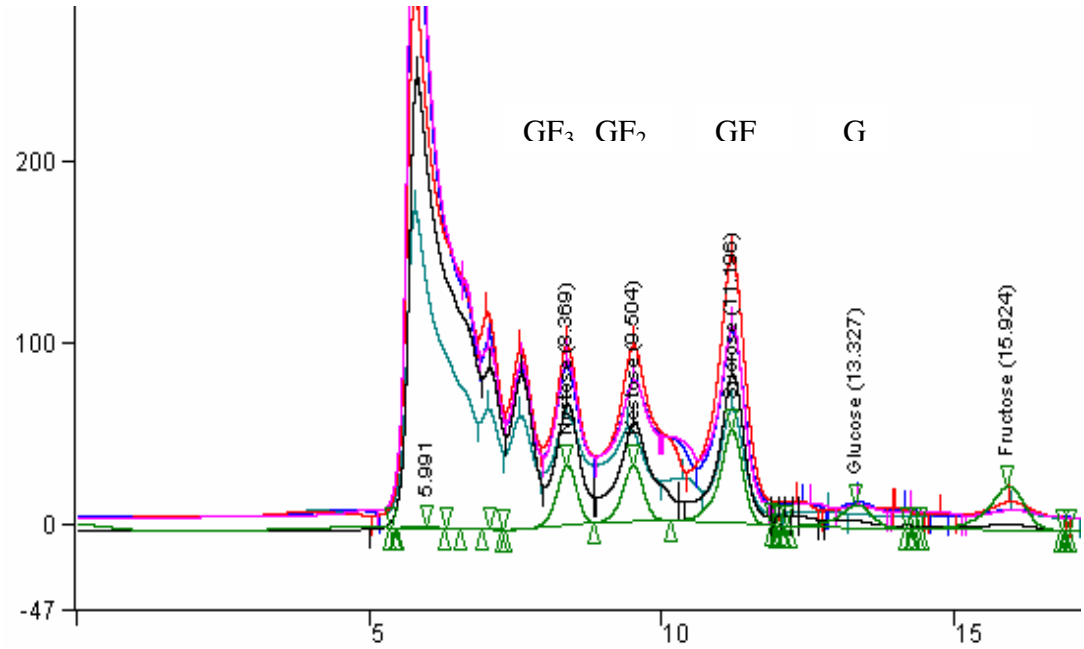


Figure E7. HPLC chromatograms of April 2006-harvested jerusalem artichoke tubers under acidic conditions. Black for fresh, pink for 5 day-stored, blue for 10 day-stored, turquoise for 15 day-stored, red for 20 day-stored, and green represents the standart.

## APPENDIX F

## COMPOSITION OF MRS BASAL MEDIUM

Peptone from casein: 10g

Yeast extract: 4g

Di-potassium hydrogen phosphate: 2g

Tween 80: 1ml

Di-ammonium hydrogen citrate: 2g

Sodium acetate trihydrate: 8.3g

Magnesium sulphate monohydrate: 0.038g

Distilled water: 1000ml

# APPENDIX G

# REPRODUCIBILITY DATA

## F1. For Water-bath Experiments

Firstly, the yield and the degree of polymerization values obtained via February 2005&2006-harvested JAs measured were compared. These values were summarized below.

Table F1.1 Results of dry-matter and Nelson-Somogyi analysis of water-bath extractions in the month February of the years 2005 &2006.

| | Yield based on 100g JA (g) | DP |
|---|---|---|
| February 2005-Fresh-NA | 13.10 | 6.57 |
| February 2006-Fresh-NA | 14.47 | 7.39 |
| February 2005-10 day stored-NA | 17.14 | 6.96 |
| February 2006-10 day stored NA | 16.53 | 6.83 |
| February 2005-10 day stored-A | 16.21 | 6.47 |
| February 2006-10 day stored A | 16.51 | 6.69 |

The differences between the yields and degree of polymerization values in these years were found as 4.9% and 5.9% for fresh-NA conditions, 0.9% and 0.9% for 10 day-stored-NA conditions, 0.9% and 1.7% for 10 day-stored-A conditions.Thus average values for yield and DP were found as 2.2% and 2.8%, respectively.

The values of yield and degreee of polymerization of the water-bath experiments done as described in Chapter 2 were summarized in Table F1.2. Sample calculations of the results can be seen in Appendix D. As can be calculated from the table, 0.9% difference between the highest and lowest values in yields obtained from dry-matter analysis, 1.3% difference in DP obtained from Nelson-Somogyi analysis. Thus, the average values used in comparisons were found as 1.6% and 2.1%, respectively

Table F1.2 Results of dry-matter and Nelson-Somogyi analysis of water-bath extractions

| | I | II | III | IV | V | AV |
|---|---|---|---|---|---|---|
| Yield based on 100g JA (g) | 10.02 | 10.01 | 9.90 | 9.94 | 9.85 | 9.94 |
| DP | 9.08 | 8.90 | 8.81 | 8.97 | 8.89 | 8.93 |

The results of HPLC analysis of water-bath extractions were given in Table F1.2. Sample calculations of these results can be seen in Appendix D. As can be calculated from the table, 0.59% difference between the highest and lowest values in DP 1-2, 0.73% in DP 3-4, and 0.82% in DP 3-6 were observed based on extracted amount of total monosaccharide units. By doing similar calculations based on jerusalem artichoke sample used in extractions, 0.48% difference in DP 1-2, 0.39% difference in DP 3-4, and 0.54% difference in DP 3-6 were obtained.

Table F1.3 Results of HPLC analysis of water-bath extractions

| | I | II | III | IV | V |
|---|---|---|---|---|---|
| Fructose (mg/ml) | 0.2800 | 0.3062 | 0.3334 | 0.3146 | 0.3723 |
| Glucose (mg/ml) | 0.3354 | 0.3266 | 0.3607 | 0.3118 | 0.3610 |
| Sucrose (mg/ml) | 5.1025 | 4.8973 | 4.7756 | 4.8989 | 4.7966 |
| Kestose (mg/ml) | 3.5197 | 3.3970 | 3.9674 | 4.3848 | 4.1551 |
| Nystose (mg/ml) | 3.6800 | 3.3242 | 3.3297 | 3.0180 | 3.1052 |
| DP5*est (mg/ml) | 0.3949 | 0.3955 | 0.4070 | 0.4140 | 0.4030 |
| DP6*est (mg/ml) | 0.4753 | 0.4694 | 0.4811 | 0.4815 | 0.4792 |

## F2. For Ultrasonic-bath Experiments

The values of yield and degreee of polymerization of the ultrasonic-bath experiments done as described in Chapter 2 were summarized in Table F2.1. Sample calculations of the results can be seen in Appendix D. As can be calculated from the table, 2.4% difference between the highest and lowest values in yields obtained from dry-matter analysis, 3% difference in DP obtained from Nelson-Somogyi analysis.

Table F2.1 Results of dry-matter and Nelson-Somogyi analysis of ultrasonic-bath extractions

| | I | II | III | IV | V | AV |
|---|---|---|---|---|---|---|
| Yield based on 100g JA (g) | 6.99 | 7.30 | 7.10 | 7.35 | 7.04 | 7.16 |
| DP | 7.49 | 7.21 | 7.59 | 7.35 | 7.53 | 7.43 |

The results of HPLC analysis of ultrasonic-bath extractions were given in Table F2.2. Sample calculations of these results can be seen in Appendix D. As can be calculated from the table, 1.52% difference between the highest and lowest values in DP 1-2, 1.82% in DP 3-4, and 2.62% in DP 3-6 were observed based on extracted amounts. By doing similar calculations based on jerusalem artichoke sample used in extractions, 0.47% difference in DP 1-2, 0.7% difference in DP 3-4, and 0.84% difference in DP 3-6 were obtained.

Table F2.2 Results of HPLC analysis of ultrasonic-bath extractions

| | I | II | III | IV | V |
|---|---|---|---|---|---|
| Fructose (mg/ml) | 0.3265 | 0.3806 | 0.3204 | 0.2946 | 0.3912 |
| Glucose (mg/ml) | 0.0392 | 0 | 0.0301 | 0 | 0.0215 |
| Sucrose (mg/ml) | 1.9941 | 1.9353 | 1.8126 | 1.8160 | 1.7641 |
| Kestose (mg/ml) | 2.0419 | 2.0303 | 2.3037 | 1.7440 | 1.9778 |
| Nystose (mg/ml) | 1.4884 | 1.4620 | 1.4970 | 1.9056 | 1.4623 |
| DP5*est (mg/ml) | 0.9970 | 0.9987 | 1.1717 | 1.0800 | 1.2160 |
| DP6*est (mg/ml) | 0.8946 | 0.9042 | 0.8613 | 0.8978 | 0.9130 |

# VITA

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## EDUCATION

- Hacettepe University, Department of Chemical Engineering, 2000
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## EXPERIENCE

- METU, Department of Chemical Engineering, 2001-2006, OYP assistant.
- AICHE, Poster presentation, San Francisco, California, November, 2006.
- Nutraceuticals and Functional Foods, Oral presentation, İstanbul, May 2006.
- 13th National Chemical Engineering Conference, Poster presentation, June, 2004.