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**PRODUCTION AND POTENTIAL APPLICATIONS OF FLOUR FROM
JERUSALEM ARTICHOKE TUBERS**

by

Nitin Dilawri, B. Sc., (Honours Biology)

**A thesis submitted to the faculty of Graduate Studies
in partial fulfillment of the requirements for the
degree of Master of Science.**

Department of Biology

Carleton University

Ottawa, Ontario.

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The undersigned hereby recommend to the Faculty of Graduate
Studies acceptance of this thesis, entitled 'Production and
Applications of Crude and Purified Flour From Jerusalem
Artichoke Tubers' submitted by Nitin Dilawri (B.Sc.
Hon., Carleton University), in partial fulfillment of the
requirements for the degree of Master of Science.

A handwritten signature in black ink, appearing to be 'RC' followed by a horizontal line and a small flourish.

Chairman, Department of Biology

A handwritten signature in black ink, appearing to be 'Nitin Dilawri'.

Supervisor

Abstract

The purpose of this thesis is the production and application of a Jerusalem artichoke (JA) flour from JA tubers which are a natural source of fructooligosaccharides (FOS) known to provide health benefits in humans by stimulating 'beneficial' intestinal bacteria, such as bifidobacteria.

Crude JA flour was produced by maceration and spray-drying of JA tubers. The administration of the JA flour supplemented milk replacer to piglets (18-24 hours old) seemed to be as effective as immunoglobulins in reducing scouring. However, more feeding trials are necessary to establish this conclusively. The crude JA flour was also found to support growth of commercially important fungi.

For potential human use, purified JA flour was produced by extraction of sliced JA tubers in a hot water mixture containing activated charcoal, followed by heating, filtration and lyophilization. The purified JA flour, rich in FOS supported good growth of bifidobacteria and therefore has commercial potential as a food ingredient to obtain bifidobacteria related health benefits.

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and Drug Administration since they wish to export the Meioligo P FOS preparation to North America for use as a food additive. However, a product of such a 'new' process will likely undergo a long and scrupulous regulatory process.

The Animal Research Centre (Agriculture Canada, Ottawa) aware of these advantages of FOS, approached Dr. Yamazaki (Caleton University, Ottawa) to prepare FOS-rich syrup for inclusion in a piglet milk replacer. Since the syrup was too costly and inconvenient for animal use, the first project in the present thesis - preparing a dry formulation of FOS (flour) from JA tubers - was initiated. The flour (dried tuber homogenate), unlike the syrup, could be more cheaply produced and more easily and stably stored. Such a flour would contain naturally produced fructooligosaccharides as well as other components of dietary value (Table 1).

Pilot plant studies were necessary for production of flour requiring collaboration of the Food Research Centre (Agriculture Canada, Ottawa) which is equipped with pulverizing, homogenizing, blending, heating, filtering, spray-drying and other equipment required for large scale processing of JA tubers.

Chapter one of the present thesis describes the production and characterization of crude JA flour from JA tubers. Chapter two discusses the potential application of crude JA flour in a piglet milk replacer and as a fungal growth medium. The crude flour had some off-flavour and odour. Thus Chapter three details the development of a process for removing colour and off-flavour

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LIST OF ABBREVIATIONS

kg	kilogram
g	gram
ug	microgram
L	litre
mL	millilitre
ul	microlitre
M	molar
m²	meters squared
ha	hectare
mm	millimetre
cm	centimetre
nm	nanometer
(v/v)	volume per volume
(w/v)	weight per volume
(w/w)	weight per weight
ca.	approximately
%	per cent
mm Hg	millimeter of mercury
JA	Jerusalem artichoke

FOS	fructooligosaccharide
BMR	basal milk replacer
O.D.	optical density
IG	immunoglobulin
min	minute(s)
°C	degrees centigrade
rpm	revolutions per minute
A _{490,510,620}	absorbance at 490, 510, 620 nm
dp	degree of polymerization
PVP	polyvinylpyrrolidone
HPLC	high pressure liquid chromatography
<u>A. niger</u>	<u>Aspergillus niger</u>
<u>P. chrysogenum</u>	<u>Penicillium chrysogenum</u>
<u>B. infantis</u>	<u>Bifidobacterium infantis</u>
<u>B. adoloescentis</u>	<u>Bifidobacterium adolescentis</u>
<u>B. longum</u>	<u>Bifidobacterium longum</u>
<u>E. coli</u>	<u>Escherichia coli</u>
DPO	diphenoloxidase
TPY	Trypticase Phytone Yeast broth for bifidobacteria

GENERAL INTRODUCTION

Due to declining demands and international competition, Canada is presently experiencing an overproduction of several conventional crops such as wheat in the Western provinces, potatoes in the Eastern provinces and tobacco in Ontario. The declining demands and increasing cost of production of these crops have been causing financial hardships to Canadian farmers, often resulting in bankruptcy. Such farmers would welcome the opportunity to grow an alternative crop which could be competitively grown in Canada and has the potential to generate new products for international markets.

Jerusalem artichoke (Helianthus tuberosus, abbreviated JA), is related to the sunflower (Helianthus annuus) earning it the nickname 'wild sunflower'. It is a perennial plant which arises from fleshy rootstocks that bear knobby (Colombia variety) or smooth oblong tubers (Challenger variety) depending on the genetic strain and harvest time (Kosaric et al., 1984). Being a native Canadian plant, it grows well in northern climates; it is resistant to most insects and diseases occurring here; is adaptable to different soils and its tubers can yield greater amounts of carbohydrates than wheat and corn. Its major carbohydrates are fructose (F) polymers (inulofructosaccharides or fructans) which consist of one terminal glucose (G) and 2-35 fructose units (GF_{2-35}) (Fig. 1). For convenience, they can be arbitrarily divided into two groups : fructooligosaccharides (abbreviated FOS) which contain up to 8 fructose residues and inulin, which contains fructose residues of 9 or greater (Stauffer et al., 1981). Other plants that produce inulofructosaccharides

m REPRESENTS THE TERMINAL SUCROSYL GROUP

n REPRESENTS THE FRUCTOFURANOSE MOIETY

[*n* = 1 — (approx.) 35]

Fig 1. Structure of inulofructosaccharides in the JA tubers. These carbohydrates are polymers of fructose with $\beta(2 \rightarrow 1)$ linkages, terminating in a glucose molecule. Degree of polymerization (dp) ranges from 2 (sucrose) to about 35 (inulin). Source (Hoehn et al., 1982).

include: chicory, dahlia, dandelion, Canada thistle, goldenrod and wild onion. However, JA tubers are the highest yielding source (Stauffer *et al.*, 1981).

Agriculture Canada studies (Hergert, 1986) show that high yields of JA tubers are obtainable in Manitoba, Saskatchewan and British Columbia. A hectare of JA produces 40-50 tons of tubers, compared to 30-40 tons/ha of potatoes, and yields 5200 kg/ha carbohydrate compared to 4500 kg/ha, and 3100 kg/ha from corn and sugar beets, respectively (Fleming and GrootWassink, 1979). The cost of the tubers to the buyers is estimated to be \$55-70/ton, or \$0.4-0.5/kg fructans in the tubers. In the Ottawa Valley, the fructan-rich tubers are harvested in late October or late April after wintering in the ground.

JA tubers were a staple vegetable for North American Indians and early settlers, and were introduced to Europeans during the 17th century. Its popularity declined with the introduction of the potato from southern regions of North America (Routley, 1977). Only recently has interest been revived in the Jerusalem artichoke because of its high carbohydrate yield.

Canadian research initially focussed on the potential of JA as a biomass crop for production of fuel grade alcohol. More recently, JA has received renewed interest as a source of fructose. Production of fructose syrup by hydrolyzing the tuber fructans was considered because fructose is the major sugar component of the tubers. Fructose is 1.3 times sweeter and less cariogenic than sucrose. Furthermore, fructose crystallizes less readily than sucrose thus giving high sugar

foods a smoother texture and chelates metal ions responsible for off-flavour (Fleming and GrootWassink, 1979). Therefore, fructose has been preferred in the food industry. Moreover, utilization of fructose does not require insulin unlike glucose or glucose-releasing sweeteners (e.g. sucrose). Thus, fructose is suitable for diabetics and calorie-conscious people who can enjoy the same sweetness with 30 % less calories.

At present, a high fructose-glucose syrup (containing 55 % fructose) is commonly used in the beverage industry. The syrup is produced via an elaborate enzymatic process: saccharization of starch by α -amylase and glucoamylase, transformation of glucose to 42 % fructose, chromatographic enrichment to 95 % fructose and blending of the two to produce 55 % fructose. In comparison, production of high-fructose syrup from JA fructans is relatively simple. A process developed by Hoehn *et al* (1982) involves extraction of the fructans from JA tuber slices, removal of small molecules by ultrafiltration, enzymic hydrolysis of the fructans; separation of fructose and glucose from high molecular weight proteins by ultrafiltration, and concentration to a syrup of 70-80 % solids. However, this method is time-consuming, limiting the amount of tubers that can be processed. Cold storage of the tubers is necessary if not used immediately because the tubers are susceptible to infection by molds. Thus, cost of tuber storage does not make production of fructose from JA tubers as attractive as that from corn starch. Moreover, commercialization of a JA-derived fructose sweetener could not overcome

competition from the sugar industries' well established production of sugars from sugar cane or corn starch, unless the JA sweetener has a unique advantage over the conventional sugars.

To reduce time and cost of tuber processing and produce a unique product, Dr. Yamazaki (Carleton University, Ottawa) has recently patented a method for production of high-fructose syrup containing fructooligosaccharides (FOS) from the JA tubers. The acid hydrolysis is shorter (only a few minutes) and less costly than the enzymic methods. The method consists of (i) slicing tubers (ii) hot water extraction of fructans (iii) passage through cation exchanger (H^+ form) to remove cations and release H^+ (iv) short heating to achieve limited hydrolysis (v) charcoal treatment (vi) filtration (vii) passage through anion exchanger (OH^- form) to remove anions and neutralize the product and (viii) concentration by reverse osmosis and evaporation. The low glucose syrup rich in FOS may be a more ideal sweetener for diabetics or calorie-conscious.

Recent Japanese studies have shown that small size FOS such as GF_{2-6} or F_{2-6} , though not utilized by humans and animals, selectively stimulate growth of 'beneficial' bifidobacteria in the intestine (Hidaka *et al.*, 1986). When bifidobacteria utilize the small-sized FOS as carbon sources, acetic and lactic acid are produced. These acids lower the intestinal pH, inhibiting the growth of 'unfavourable' intestinal bacteria such as Escherichia. coli and Clostridium perfringens which produce toxic

bad-smelling substances such as NH_3 , amines (that can react with nitrate or nitrite forming carcinogenic nitrosamines), H_2S , skatole and indole (Hidaka et al., 1986). In addition, the acids retard intestinal absorption of ammonia and amines by protonation, stimulating bowel movement. Bifidobacteria provide hosts with vitamins (B_1 , B_6 , B_{12} , pantothenic and nicotinic acids), degrade nitrosamines, and stimulate intestinal immunity against infection (Deguchi et al., 1985, Yamazaki et al., 1985). Decline of the bifidobacteria population is commonly observed in unhealthy or elderly human hosts (Mitsuoka, 1982). Clinical studies have shown that FOS administration increases bifidobacteria population; reduces the population of 'unfavourable' bacteria; reduces constipation, blood lipids in hyperlipidemia, blood pressure, blood cholesterol and production of intestinal toxic substances (Hidaka et al., 1986).

Meiji Seika Kaisha Ltd. of Japan has commercialized production of small-sized FOS from sucrose by the action of a fungal *B*-fructofuranosidase ($\text{GF} \rightarrow \text{GF}_1 + \text{GF}_2 + \text{GF}_3 + \text{GF}_4 + \text{GF}_5$) derived from Aspergillus niger. The FOS preparation (Meiologo P) is now used in Japan as an ingredient in human food (e.g. drinks, confectionaries, preserves, and dairy products); in pet food to reduce fecal odour and in piglet feed to reduce diarrhea, promote weight gain and feed efficiency (presumably by stimulating the growth of the 'beneficial' bacteria) (Hidaka et al., 1986). Meiji Seika Kaisha Ltd. is now seeking the approval of the U.S. Food

and Drug Administration since they wish to export the Meioligo P FOS preparation to North America for use as a food additive. However, a product of such a 'new' process will likely undergo a long and scrupulous regulatory process.

The Animal Research Centre (Agriculture Canada, Ottawa) aware of these advantages of FOS, approached Dr. Yamazaki (Caleton University, Ottawa) to prepare FOS-rich syrup for inclusion in a piglet milk replacer. Since the syrup was too costly and inconvenient for animal use, the first project in the present thesis - preparing a dry formulation of FOS (flour) from JA tubers - was initiated. The flour (dried tuber homogenate), unlike the syrup, could be more cheaply produced and more easily and stably stored. Such a flour would contain naturally produced fructooligosaccharides as well as other components of dietary value (Table 1).

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Table 1. Main components contained in Jerusalem artichoke tubers.

COMPOSITION		
Component	Fresh weight (%)	Dry weight ^(a) (%)
Water	80	-
Carbohydrates	15.2-28.8	68.4-83.1
Proteins	0.29-0.31	1.45-1.55
Lipids	0.1	0.5
Ash	1.1	4.7
Cellulose and Hemicellulose	2.62	13.1

(a) 20 % of tuber weight are solids.

Source : (Flemming and GrootWassink, 1979).

to produce a FOS flour fit for human consumption. Chapter four demonstrates that the purified JA FOS preparation supports good growth of bifidobacteria. This Chapter also includes an investigation into the utilization of the purified JA FOS preparation by Saccharomyces cerevisiae since this information would be valuable for future application of this product in leavened products.

CHAPTER ONE

PRODUCTION OF CRUDE JA FLOUR FROM JERUSALEM · ARTICHOKE TUBERS

Introduction

A major problem in commercialization of JA tuber products is that the fresh tubers are not available year round in this region. Year-round production would require storage of the tubers (refrigeration) which is expensive in terms of capital and the requirement of storage space and transport of the tubers into and out of storage. Dehydrated tuber slices can be stored at room temperature. However, the drying process is time-consuming: heating the sliced tubers 50-80°C for several hours. Since we propose here to use JA tubers in a piglet milk replacer, a powdered product (JA flour) is desired since it is miscible in aqueous formula. Currently, JA flour is produced by 'hammer' milling the dried slices. This method is energy intensive. Our proposal for producing the JA flour, consists of maceration of fresh tubers and spray-drying of the homogenate. The process should be faster than the existing method and the sufficiently dried flour could be stably stored at room temperature. This chapter describes production of crude flour from whole JA tubers.

Results and Discussion

To determine the composition of the water-soluble fructooligosaccharides (FOS) in the JA tubers, the JA tubers were minced in one part of water and filtered. HPLC (high pressure liquid chromatography) analysis of this filtrate revealed that the FOS composition was as follows: in % dry weight; in terms of

degree of polymerization, (dp): dp=1+2, mono- and di-saccharides (fructose or glucose plus sucrose (GF) or F_2 , ca. 20; dp=3-6 (GF_{2-5} or F_{3-6}), ca. 50; dp>6 ($GF_{>5}$ or $F_{>6}$), ca. 30. The dp 1+2 fraction is utilizable by humans and provides most of the sweetness in the tubers (since sweetness declines as dp increases). Recently, Japanese researchers have shown that dp 3-6 FOS (e.g. GF_{2-5}) contained in Meioligo P (which is enzymically derived from sucrose) stimulate growth of 'beneficial' intestinal bacteria to produce desirable dietary effects (Hidaka *et al.*, 1986). Meioligo P has also been shown to be used to reduce diarrhea and increase weight gain and feed efficiency of two-week old piglets (Dr. Hidaka, personal communication, 1988). How well the longer chain FOS (dp>6) can support these 'beneficial' bacteria (bifidobacteria in humans) was not known at the time of initiation of this thesis. Therefore this will be addressed in Chapter four. In the present investigation, an attempt was made to produce JA flour with a high content of dp 3-6 FOS. During JA tuber processing, there is a potential for enzymic browning reactions (associated with production off-flavour and aroma) to occur since the tubers contain an abundance of phenolic compounds (Paupardin, 1968) which could be oxidized by endogenous diphenoloxidases (abbreviated, DPO). Since the JA flour is to be used in a piglet milk replacer or possibly as a food additive for humans, the production process should minimize development of colour and off-flavour.

In the first set of JA flour production trials (Lot 1-5; Table 1.1), washed tubers were pulverized (dicing and macerated), passed through a plate type heat exchanger at 100-140°C (for 20 min) to examine its effect on discolouration (since DPO may be denatured upon heating) as well as FOS composition and then spray-dried at 180°C (Fig 1.1). For each lot, 100 kg of tubers were used since the minimum amount of macerated tubers that could be spray-dried was 100 kg. Each lot weighed ca. 20 kg. It was observed that colour was being largely generated during maceration. However, heating at 100-120°C seemed to prevent further development of colour whereas heating above 120°C (e.g. 130 or 140°C) generated more colour presumably because of sugar caramelization. The effect of heating on the FOS composition of the tubers was determined by analyzing the water soluble FOS contained in the lots of flour by HPLC. Table 1.1 (Lots 1-5) shows that increased heating of the tuber homogenate caused greater hydrolysis of the higher molecular weight fraction ($dp > 6$), generating more mono- and di-saccharides, resulting in increased sweetness of the flour. More importantly, HPLC analyses show that the desirable length dp 3-6 FOS fraction in the tubers also decreased upon heating.

In the next set of flour production trials (Table 1.1; Lot 6-12) we wished to examine the effect of mild acid treatment (0.01 parts glacial acetic acid was added

Table 1.1. The effect of heating and acidification on the FOS composition of the crude JA flour (a).

LOT #	TEMPERATURE (°C)	FOS (% dry weight) ^(b)		
		dp = 1+2	dp = 3-6	dp > 6
1	100	24.4	42.7	32.9
2	110	30.0	41.0	29.0
3	120	39.5	37.8	22.7
4	130	50.8	34.0	15.2
5	140	63.5	31.2	5.3
6	100 (i)	26.5	43.4	30.1
7	100 (ii)	27.0	43.0	30.0
8	110 (i)	32.0	42.4	25.6
9	110 (ii)	30.3	42.2	27.5
10	120 (i)	38.1	41.4	20.5
11	120 (ii)	40.1	38.1	21.8
12	25 (i)	28.5	43.4	28.1
13	25(no acid)	20.1	47.0	32.9

- (a) Lot 1-5 were macerated and heated at 100-140°C for 20 min and spray-dried, while for Lot 6-12 0.01 parts acetic acid was added before (i) or after (ii) maceration, heated at 100-120°C for 10 min and then spray-dried. Lot 12 and 13 are control batches Lot 12, acid only; an Lot 13, no heat or acid.
- (b) HPLC analysis of the lots of flour resolved oligosaccharides up to dp=6 (dp, refers to degree of polymerization e.g. dp=1+2, glucose or fructose plus sucrose (GF) or F₂; and dp=3-6, GF₂₋₅ or F₃₋₆; and dp>6, GF_{>5} or F_{>6}. Results are expressed as the average % dry weight of FOS calculated from duplicate samples from each lot of flour.

Fig 1.1. Flow diagram for production of flour from whole JA tubers. In the first set of production trials (Lot 1-5) the tuber homogenates were heated at 100-140°C for 20 min. In the next set of production trials (Lots 6-11) 0.01 parts acetic acid was added to 1 part of tubers before (i) or after (ii) maceration and the tuber homogenates heated at 100-120°C for 10 min.

to 1 part tuber) which may hydrolyze the $dp>6$ fraction in the tubers and generate desirable dp 3-6 FOS. Two modes of acidification were used: before or after maceration of the tubers, to observe the effect of acid treatment on discolouration (since lowering the pH of the tuber homogenate (pH ca. 6.0) may facilitate inactivation of DPO). The acidified tuber homogenates (Lots 6-11; pH ca. 4.3) were heated at 100-120°C for 10 min. Control lots of JA flour consisted of the spray-dried tuber homogenates with acid treatment but no heating (Lot 12) and without acid and heat treatment (Lot 13; pH ca. 5.1). HPLC analyses of Lots 6-9 (Table 1.1) show that heating the acidified homogenates at 100°C and 110°C caused a slight increase in the $dp=3-6$ fraction relative to the $dp=3-6$ content of the homogenates heated without acid at 100°C and 110°C (Lots 1 and 2). Heating the acidified tuber homogenate at 120°C (Lots 10 and 11) caused increased hydrolysis of the $dp>6$ fraction and some hydrolysis of the $dp=3-6$ fraction, resulting in increased mono- and di-saccharides (relative to the acidified lots heated at 100°C and 110°C; Lots 6-9). However, the spray-dried tuber homogenate without any treatment (no acid and no heat; Lot 13) contained the highest levels of desirable length FOS ($dp=3-6$; ca. 47 % dry weight of FOS in the tubers) though the flour was somewhat darker. Although colour was largely generated during maceration (before heat treatment) the addition of acid before maceration was found to

prevent further development of colour.

These preliminary JA flour production trials, showed us that acid treatment will not significantly increase the dp 3-6 FOS fraction in the tubers and that heating of the JA tuber homogenate (100-110°C) or acidification before maceration may minimize further development of colour. However, for the production of JA flour with minimum discolouration, maceration should be avoided or air should be expelled during maceration. Use of steam injection during maceration has been proposed (Yamazaki *et al.*, patent pending).

Lots 1-3 (tuber homogenate that was heated from 100-110°C and then spray-dried) and Lot 13 (spray-dried tuber homogenate) were blended, yielding ca. 80 kg of flour, and used as a source of FOS for examination in a piglet milk replacer (Chapter two). The main components of the blended flour were analyzed as follows: FOS composition of the blend was determined by HPLC; protein content was determined using the Kjeldahl method; total ash was determined using the method in Umbreit *et al.* (1972); the amount of insoluble components was estimated by filtering a solution of the blended flour and weighing the dried insoluble material collected; and finally, the % dry weight of soluble carbohydrates was estimated by adding total protein, ash and insoluble components (in % dry weight) and subtracting from 100, since soluble carbohydrates are the major soluble component in JA tubers (Table 1). Details of the composition analyses of the blended flour are given in the methods section. Fig 1.2 summarizes the composition of the blended

flour.

We provide a rapid method for production of JA flour which can rapidly process a large amount of tubers within 20 min, eliminating the need for costly tuber storage. The production of JA flour should facilitate commercialization of JA tubers, making them a more attractive alternative crop for farmers.

Fig 1.2. FOS composition of crude JA flour blend to be used in piglet milk replacer and as growth medium for fungi. All of Lots 1-3 and 13 (ca. 20 kg/lot) were blended. FOS composition was determined by analyzing a 2 % (solids) solution of the blended flour by HPLC as described in methods. Numbers above the peaks refer to degree of polymerization (dp) and the order of elution of oligosaccharides is from the top of the chromatogram (dp>6) to the bottom (dp=1). Percent of each oligosaccharide present was calculated from the proportion of total area which each peak occupies. Composition is as follows (in % dry weight): mono- and disaccharides dp=1+2; 33.3, FOS dp=3-6; 46.4; and FOS dp>6; 20.3. Component composition (in % dry weight): soluble carbohydrates; ca. 77.5, protein; 2.1, ash; 4.2, and insoluble components; 16.2.

FOS COMPOSITION (%)

dp = 1	13.6
dp = 2	19.7
dp = 3	13.9
dp = 4	12.2
dp = 5	11.9
dp = 6	8.4
dp > 6	20.3

Materials and Methods

Materials

Jerusalem artichoke tubers (Colombia variety) used in this study were harvested in late October 1987 and supplied by Agriculture Canada, Ottawa, Ontario. Approximately 1.3 tonnes of tubers were processed in a pilot plant set-up at the Food Research Centre, Agriculture Canada. Details of equipment cannot be disclosed because it is patent pending. All chemicals employed were of analytical grade and obtained from the Sigma Chemical Company, St. Louis, Mo., U.S.A.

Methods

Pilot plant production of crude JA flour from JA tubers

A pilot plant set up at the Food Research Centre was used for flour production. Fig 1.1 shows the major steps in the flour production process. A minimum of 100 kg of JA tubers (Colombia type) were used for each lot.

In the first (Lot 1-5) production trials macerated tubers were heated from 100-140°C for 20 minutes and then spray-dried. In the next trials (Lots 6-11) heating (100-120°C) for 10 minutes was combined with acid treatment in which 0.01 parts glacial acetic acid was added to the tubers ; (i) before or (ii) after maceration; followed by spray-drying. Control lots consisted of spray-dried tuber homogenates with acid treatment but no heating (Lot 12) and without acid or heat treatment (Lot 13).

Oligosaccharide analysis

All oligosaccharide analyses were performed at Agriculture Canada (Food Research Centre) using a Perkin-Elmer Series 4 (IS-100) HPLC fitted with two TSK-Oligo (#1172) columns (Aitzetmuller, 1978) with Meioligo P used as the standard (both supplied by Meiji Seika Kaisha Ltd. of Japan). The solvent system used was a ethyl formate-methanol-water (1:2:1) solution (Aitzetmuller, 1978). These columns allowed separation of oligosaccharides through $dp=6$.

All samples were prepared as follows:

2.5 g of crude flour from each lot was suspended in 50 mL of double distilled water. Samples were squeezed through a nylon mesh (10 μ m pore size, supplied by Meiji Seika Kaisha Ltd.). Using a hand refractive index meter (Atago Co. of Japan; N1 #2110-W04)) % solid in the sample was determined by placing 200 μ L of the sample on the meter and reading the scale. Samples were diluted with double distilled water to 2 % solids (w/v)(pH was determined at this step using a hand pH meter (Atago Co. of Japan; #2201-P01), passed through a 0.45 μ m (Sartorius) filter into a HPLC vial (1.8 mL). 20 μ L of each sample was automatically loaded on the column and analyzed within 30 minutes. The proportion of oligosaccharides (relative concentration of each oligosaccharide) present, were expressed as % of the total area (% dry weight); calculated automatically by an attached integrator.

Determination of total ash

A 7 cm diameter silica dish was heated by a bunsen burner for 1 min and then cooled and weighed. 2 g of the crude JA flour blend was then heated gently on a bunsen burner in the silica dish for 5 min. The dish was then partially covered with tinfoil and transferred to a 400°C furnace for 4 hours. The dish was then transferred to a dessicator, cooled and weighed. Average total ash was calculated from three separate 2 g samples, as the percentage of weight remaining from the original sample (Umbreit et al., 1972).

Determination of total protein

Nitrogen in the crude JA flour was determined using a modified Kjeldahl method using a Tecator Digestion System (# 20-1015) and a Kjeltec Auto 1030 Analyzer (Tecator Systems, Hoganas, Sweden, Box 70, S-263 01).

Two 0.2 g samples of JA flour were transferred to separate digestion tubes containing Kjeltab (Hg, Se, Cu) catalysts and mixed gently in 5 mL conc. H_2SO_4 . The tubes were then heated to 420°C in a Tecator digestion rack for 4 min. The tubes were then cooled and diluted by adding 25 mL of distilled water and loaded onto the Kjeltec Auto Analyzer. The solution was then automatically titrated with 1 N HCl and % nitrogen calculated automatically. % nitrogen was converted to % protein by multiplying by 5.9 (conversion factor for plant material) (Kjeltec Auto

Analyzer Manual, 1985). Average % protein was calculated by repeating this with another sample of the blended JA flour.

Determination of the insoluble component

100 g of crude flour was dissolved in 500 mL of distilled water passed through two layers of cheesecloth and the pulp placed on a pre-weighed pre-dried Whatman #1 and dried at 100°C for 3 hours (rinsings from cheesecloth were added to the filtrate). The filtrate was filtered through a pre-weighed and dried Whatman #1 and then dried (100°C) for 3 hours. % (dry weight) of insoluble material was then calculated by adding the dry weights of the samples collected and dividing by 100. The average insoluble component (% dry weight) was calculated by repeating this with two more samples of the blended flour.

Determination of the total soluble carbohydrate content

The remaining component in the flour was mainly soluble carbohydrate (Table 1; General Introduction) which was estimated by adding the average protein, ash and insoluble components and subtracting the total from 100.

CHAPTER TWO

APPLICATION OF CRUDE JA FLOUR IN A PIGLET MILK REPLACER AND AS A FUNGAL GROWTH MEDIUM

Introduction

For the baby piglet, the sow's milk not only supplies nutrients, but also immunoglobulins (Aumaitre and Seve, 1978). Immunoglobulins are in high concentration in the sows' first milk (colostrum) after birth. The baby piglet is born with no functioning immune system, thus the immunoglobulins it receives via the colostrum are essential for the newborn piglet's defence against infection and disease until its own immune system starts to function 10-14 days after birth (Wilson, 1974). Under conventional rearing situations, piglets receive mother's milk for about 4-6 weeks, and then are weaned onto a solid diet.

In spite of this, piglet mortality during the suckling period remains one of the major problems facing swine producers. Although values of deaths of pre-weaned piglets of 10 % (piglets in a litter dying before weaning because of the inability of the piglet to obtain sufficient immunoglobulin-rich milk) are often quoted, however, losses of up to 25 % on Canadian farms can be found (Lang, 1987). This results from problems at lactation, extra large litters, competition within the litter or poor nursing sows (Dr.Farnworth, personal communication, 1988). In recognition of this problem, the Animal Research Centre (ARC) branch of Agriculture Canada has made reduction of piglet mortality a priority.

The development of milk replacers for domestic animals has been an area of research for some time (McRoberts and Hogan, 1944, Lecce, 1975). This has arisen because of the desire to raise piglets away from the sow for experimental

purposes and because of the situations where swine producers might not want or are unable to leave newborn animals with their mothers (Farnworth and Elliot, 1987). The ideal milk replacer would mimic the mother's milk as closely as possible, and thereby provide all nutrients and immunoglobulins required for growth and health.

At the ARC, housing management and diets have been developed to allow for raising newborn (a few hours old) piglets (Farnworth and Elliot, 1987). Extensive feeding trials showed that as the time on the sow decreased, mortality of piglets fed artificial milk usually increased due to diarrhea (mainly caused by enterotoxigenic E.coli). Transmissible gastroenteritis virus and rotavirus are two other enteropathogens responsible for causing neonatal diarrhea (Leece, 1986). It was found that diarrhea and death of piglets could be overcome by using a milk replacer containing an immunoglobulin preparation (Elliot et al., 1987). However, the immunoglobulin preparation is expensive since it is prepared from pigs blood using a two-step ammonium sulfate fractionation process that requires specialized equipment (e.g. centrifuge, electrodialyzer and a spray-drier) (Elliot et al., 1987).

Bouts of diarrhea which cause ill-health and weight loss, if not death are a recurrent problem during and after weaning (transition from a milk replacer or mother's milk to a solid diet). In some countries (e.g. U.S. and Japan) antibiotics are added to milk replacers and in the solid diet of post-weaned animals to protect them from bacterial infection. However, antibiotic administration retards

natural propagation of 'beneficial' intestinal bacteria (Lactobacillus and Streptococcus in piglets, inherited from mother at low densities) (Stanier *et al.*, 1986). These bacteria would help crowd-out or inhibit growth of disease causing bacteria (e.g. E. coli). In Sweden, Lactobacillus and Streptococcus are added to piglet milk replacers and starter feed. However, exogenously administered bacteria seldom colonize the intestine of recipient animals (Raibaud *et al.*, 1980). In Canada, antibiotic use is banned so we must find another way to reduce diarrhea and improve health of piglets. One possible solution is to improve the diet which may encourage growth of the 'beneficial' intestinal bacteria.

In Japan, piglets which received sow's milk and antibiotics for the first two weeks of life were transferred to a milk replacer containing mostly small-sized FOS (Meioligo P). This regimen significantly reduced scouring (diarrhea) and promoted weight gain and feed efficiency (weight gain/feed consumed). These effects were associated with an increase in population of the 'beneficial' intestinal bacteria which specifically utilize the small-sized FOS contained in Meioligo P (Hidaka *et al.*, 1986).

In Chapter one we prepared a blend of crude JA flour which contains in % dry weight of FOS in terms of degree of polymerization (dp): mono- and disaccharides 30; small-sized FOS (dp=3-6), 46 and FOS with dp>6, 24. Now, we wish to examine if the addition of this flour to a piglet milk replacer can reduce scouring and improve growth of piglets presumably by stimulating 'beneficial' bacteria, and thereby reduce the piglet's dependency on immunoglobulins in

mother's milk or in the milk replacer developed by Farnworth and Elliot (1987).

Results and Discussion

To date, administration of FOS has been restricted to piglets that were exposed to their mothers for at least two weeks (Hidaka personal comm., 1988). In this investigation, a JA flour-containing milk replacer is fed to younger animals (i.e. 0-36 hours old). Piglets were fed a basal milk replacer (Table 2.1) supplemented with an immunoglobulin preparation (+IG) (Elliot *et al.*, 1987) or the crude JA flour blend (+JA) (prepared in Chapter one) as described in Table 2.2. Feeding experiments were carried out at the ARC minimum diseases facility which prevents entry of pathogens from the outside. This place was equipped with an automated feeding system which allowed for simultaneous feeding of controlled amounts of the different test diets dispensed at regular time intervals (i.e. every two hours, to simulate the natural feeding interval) and allowed for the collection of feces (Fig 2.2; Methods). However, sample size was limited because each system one for each diet, could only house 6 animals. Thus the trends observed are suggestive, not conclusive.

The first feeding trial was intended as a preliminary investigation to see if piglets fed the JA-containing milk replacer showed any sign of gaining a benefit (i.e. reduced scouring, or increased weight gain and feed efficiency). In this experiment, 18 piglets randomly selected from two litters were exposed to their mother for 24-

Table 2.1. Dry matter constituents of the basal milk replacer (control diet)^(a).

INGREDIENTS	% DRY WEIGHT
Low temperature skim milk powder	59.92
Casein	5.95
D,L-Methionine	0.36
Vegetable oil	29.76
Vitamin pre-mix ^(b)	1.19
Mineral pre-mix ^(c)	1.19
Calcium chloride	.20
Soy lecithin	1.34
Tween-80	<u>0.09</u>
	100.00

(a) Dry matter constituents were mixed with water at the time of reconstitution.

(b) provides per kg of milk replacer: vitamin A, 5770 IU; vitamin D, 520 IU; vitamin E, 22 IU; vitamin K, 4.84 mg; thiamin, 3.19 mg; biotin, 280 ug; riboflavin, 6.6 mg; niacin, 48.4 mg; calcium pantothenate, 62.7 mg; pyrodoxine, 4.0 mg; vitamin B₁₂ 48.4 ug; choline chloride.

(c) provides per kg of milk replacer : iron 125 ppm; copper 10 ppm; manganese 20 ppm; zinc 100 ppm; iodine 0.2 ppm; selenium 0.3 ppm.

Table 2.2. Test diet formulations (a).

INGREDIENTS	BASAL IMMUNO- JA FLOUR GLOBULIN BLEND		
	<u>% OF DRY MATTER CONSTITUENTS</u>		
Basal Milk Replacer	84	84	84
Immunoglobulins	-	16	-
Skim milk	16	-	13.5
Crude JA Flour	-	-	2.5

- (a) The basal milk replacer was supplemented with 16 % (w/w) immunoglobulins or 2.5 % (w/w) crude JA flour was reconstituted in an appropriate amount of water and immediately put into the appropriate reservoir of the automatic feeding systems used (Fig 2.2; Methods). 0.1 % (v/v) formaldehyde was added to all the liquid feed as a preservative.

36 hours and then transferred to the automatic feeding systems and fed the two test milk replacers (+JA, +IG) and the basal milk replacer as the control for six days. Piglets received 9 % of their body weight of liquid feed each day which approximates their natural daily intake if they were left on the sow (Farnworth as per comm., 1988). 1/12 of the daily allocation of feed was dispensed every two hours. Feces were scored daily on a scale of 0 to 2 according to appearance and consistency such that a lower score indicates less scouring (Table 2.3). Piglets were weighed every three days and at the end of the experiment, % weight gain ($100 \times \text{weight gain (g)}/\text{initial weight}$) and feed efficiency ($\text{weight gain}/\text{total feed consumed (in dry weight)}$) were calculated. In addition, % total feed consumed was determined for each animal as follows: $100 \times \text{total dry feed eaten}/\text{total dry feed given}$ which was determined by measuring the amount of liquid feed left in their bowls each day for six days as described in Table 2.4.

Table 2.4 (Experiment 1) shows that feces scores for piglets consuming the +JA and +IG milk replacers were similar (ca. 0.5) and lower than the control group (ca. 0.8). The +JA group ate less feed; consuming ca. 12 % less feed than the control or +IG groups. This may have been due to the taste or aroma of the JA replacer which may have affected their appetite. This explains the accompanying lower weight gain (+JA group weighed ca. 5 % lower than the control group and 16 % lower than the +IG group after the six days). However, the feed efficiency of the +JA group was similar to the +IG group (ca. 1.2) and higher than the

Table 2.3. Feces scoring system used to quantitate the severity of diarrhea of piglets during the feeding trials.

<u>Score</u>	<u>Description of Feces</u>
-	No Feces
0	Normal- dry, well formed
0.5	Pasty
1	Pasty and somewhat runny
2	Watery, runny (diarrhea)

EXPERIMENT 1

Table 2.4. The effect of feeding the JA-containing milk replacer on feces scores, weight gain, feed consumption and feed efficiency of 24-36 hours old piglets (a).

	TREATMENT		
	CONTROL	+ IG	+ JA
FECES SCORES	0.80 $\pm$ 0.25	0.50 $\pm$ 0.15	0.5 $\pm$ 0.21
WEIGHT GAIN (%) ^(b)	51 $\pm$ 10	62 $\pm$ 9	46 $\pm$ 13
FEED CONSUMED (%) ^(c)	94.0 $\pm$ 0.5	96.2 $\pm$ 0.4	83.9 $\pm$ 0.3
FEED EFFICIENCY ^(d)	1.10 $\pm$ 0.02	1.21 $\pm$ 0.03	1.17 $\pm$ 0.04

(a) Eighteen (6 per diet) piglets 24-36 hours old were fed the three milk replacers : Control, Basal milk replacer (BMR); + IG, BMR with 16 % (w/w) immunoglobulin added and +JA, BMR with 2.5 % (w/w) crude JA flour blend added, for six days. All results are expressed as mean values $\pm$ standard deviation, based on a sample size of 6 piglets per treatment.

(b) % weight gain = 100 X (final weight - initial weight / initial weight).

(c) % of total feed consumed = 100 X (total liquid feed eaten - total liquid feed given/ total liquid feed given).

(d) feed efficiency = final weight - initial weight / total feed consumed as shown above.

control group (ca. 1.1), suggesting that the JA-containing milk replacer might promote weight gain if the piglets ate more.

Since all the piglets tested survived with 24-36 hours exposure to the sow, we were interested to see if piglets which were exposed to the sow for a shorter period of time to the sow, could survive on the three diets (control, +IG and +JA). Therefore, using the same regimen as in Experiment 1, the healthiest looking piglets from two litters were used since we were expecting some mortality this time. These 18-22 hours old piglets were transferred to the three test diets (6 piglets per diet) which were fed for nine days. This time, piglets consuming the JA-containing milk replacer showed a much more significant reduction in feces scores, even lower than the +IG group (Experiment 2; Table 2.5). Once again, % weight gain and % feed consumed were lower for the +JA group. The +JA group consumed ca. 8 % less feed than the control and +IG groups and gained ca. 7 % less weight than the control; and ca. 23 % less than the +IG group presumably because piglets did not seem to find it as palatable. Since the feed efficiency of the +JA group was lower than the +IG and control groups this time (Table 2.5), the suggestion that the JA-containing milk replacer might improve feed efficiency (Experiment 1; Table 2.4) is still inconclusive. No deaths occurred although feces scores for the control and +IG groups were higher than the scores in Experiment 1 indicating more severe

EXPERIMENT 2

Table 2.5. The effect of feeding the JA-containing milk replacer on feces scores, weight gain, feed consumption and feed efficiency of 18-22 hours old piglets ^(a).

	TREATMENT		
	CONTROL	+ IG	+ JA
FECES SCORES	1.11 $\pm$.35	0.89 $\pm$.26	0.52 $\pm$.22
WEIGHT GAIN (%)	133 $\pm$ 15	149 $\pm$ 12	126 $\pm$ 17
FEED CONSUMED (%)	95.5 $\pm$ 1.4	96.7 $\pm$ 0.8	87.9 $\pm$ 1.0
FEED EFFICIENCY	1.10 $\pm$.06	1.22 $\pm$.03	1.08 $\pm$.04

(a) 18 (6 per diet) piglets 18-22 hours old were fed the three test diets for 9 days. (treatment, % weight gain, feed efficiency and % feed consumed are as outlined in Table 2.4). All results are mean values $\pm$ standard deviation based on a sample size of 6 piglets per treatment.

scouring. The survival of the piglets was most likely due to the environment and feeding regimen used.

Thus, for purely academic reasons we were interested to see how well the piglets could survive on the JA replacer, if exposure to the sow was limited further (e.g 0-8 hours after birth) in this environment. Since the effectiveness of the IG-containing milk replacer had already been established for piglets this age (Elliot and Farnworth, 1978), JA and control diets were fed in Experiment 3.

Table 2.6 shows a composite of two experiments. In the first experiment, 14 piglets from two litters up to 8 hours old (the sows gave birth during the night) were transferred to the +JA and control diets (7 piglets per diet). In the second experiment, two sows given a prostaglandin piglets injection (which induces delivery) were placed in a raised box so that when piglets were born several hours later they could not obtain mother's milk. 16 piglets from two sows were collected and transferred to the test formulations (8 piglets per diet). Data from these two experiments were combined because feces scores and mortality were similar (Table 2.6).

More than half of the thirty animals tested died within the first week and none survived for more than ten days. Post-mortems indicated severe dehydration, full stomachs, and large quantities of runny feces in the colon; all of which are associated with neonatal diarrhea in piglets (Dr. Farnworth, personal communication, 1989). While alive, the +JA group had reduced feces scores (+JA,

Table 2.6. The effect of feeding the JA-containing milk replacer on survival time (mean number of days alive) and the feces scores of 0-8 hours old piglets (a).

TREATMENT	MEAN # OF DAYS ALIVE	FECES SCORES
CONTROL	7 $\pm$ 3	1.60 $\pm$.31
+ JA	6 $\pm$ 4	1.09 $\pm$.27

- (a) Data is a composite of two experiments. First, 14 piglets exposed to the sow up to 8 hours were tested on the JA and control diets (7 per diet). Then 16 newborn piglets with no exposure to the sow were tested (8 per diet). Therefore mean days alive and feces scores are based on a sample size of 15 for each diet. More than half the animals tested died within a week and all animals were dead within 10 days.

ca. 1.09; control, ca. 1.60), suggesting that the +JA diet may reduce the severity of diarrhea. However, it did not improve their chances of survival since the mean number of days alive for the control and +JA groups were similar (ca. 6-7 days). Thus the basal or JA supplemented milk replacers would not be adequate for the raising of newborn piglets which received little colostrum. Weight gain, feed consumption and feed efficiency were not calculated due to the ill-health of the piglets.

The preliminary feeding of the JA-containing milk replacer to piglets suggest that addition of the JA flour to their diet will not replace the immunoglobulins in mother's milk or in the milk replacer. However, JA flour given to piglets a few days old seems to be as effective as immunoglobulins in reducing scours and supporting growth. However, more feeding trials are necessary to establish this conclusively. If proven effective in reducing scours and supporting growth, the JA-containing milk replacers could be used by farmers in a conventional barn. However, piglets on a farm may need to be left with the mother for as long as possible (e.g. at least a week) to insure they receive an adequate supply of immunoglobulins (since scouring is expected to be more severe here) before they are transferred to a JA milk replacer. Furthermore, older piglets may not be as sensitive to the off-flavour or aroma contained in the crude JA flour. The use of the JA milk replacer may facilitate earlier removal of piglets from their mother, which may induce earlier pregnancy of the sow, accelerating pork production. Future applications of JA

products will not only help swine producers who benefit from the lower cost of the milk replacer but also farmers who could grow JA as an alternative crop.

Development of a dried preparation of JA slices for use in solid pig feed

In the near future, the ARC will be investigating the feeding of JA to post-weaned piglets (4-6 weeks old) who may not be so sensitive to its aroma or taste in hope that it may reduce scouring, increase weight gain and increase feed efficiency. In Japan, dp 3-6 FOS (Meioligo P) have been used to reduce the odour of feces in dogs since 'unfavourable' intestinal bacteria producing putrefactive compounds are suppressed by growth of 'beneficial' bacteria. Therefore, it is possible that by feeding JA products to older piglets that pig barn odour may be reduced. For such an application, the flour preparation would not be suitable since older animals prefer a granular solid diet. Thus, we suggest a method for drying 'thinly' sliced JA tubers (e.g. 0.25 cm thick). The dried thin slices could be easily crumbled and added to solid pig feed. Drying the thin slices at high temperatures may inactivate diphenoloxidase which is responsible for browning reactions (associated with off-flavour and aroma) (Chapter three; Fig 3.1).

To produce such a preparation, 100 g batches of 'thinly' sliced JA tubers (0.25 cm thick) were heated at 60°, 80° and 100°C, until dried and then ground. To determine the FOS composition the product was further ground into a powder, suspended in distilled water (0.5 g of powder/ 10 mL), and filtered. The filtrate was then analyzed for colour at A_{490} (Chapter three; Methods) and FOS

composition by HPLC. Colour and FOS composition of the JA flour blend was determined similarly for comparison (Table 2.7).

Table 2.7 shows that heating at 100°C reduced drying time although the slices were burnt. However, this resulted in a 30 % increase in colour (which is associated with off-flavours) relative to the slices heated at 60°C and 80°C. Higher temperature caused more hydrolysis of the dp 3-6 and dp>6 fractions resulting in increased mono- and di- saccharides. Drying at 60-80°C should provide an effective means of producing dried FOS-rich JA slices which would contain ca. 55 % dp 3-6 FOS with minimum discolouration (less colour than the crude JA flour blend) that could be easily crumbled and added to solid pig feed. Thus, we provide a simple drying protocol for preparing dried JA slices which could be used to test the dietary benefits of JA FOS in older piglets.

Application of the crude JA flour as a fungal growth medium

It was noticed that molds frequently appeared in solutions containing the crude JA flour which had been left on the laboratory bench for a few days. Since it is known that many fungi can degrade inulin and FOS since they possess *B*-fructosidase. Therefore I thought that the crude JA flour produced may provide a relatively cheap alternative growth medium which might support good growth of

Table 2.7. The effect of drying temperature on the colour and FOS composition of JA tuber slices.

DRYING CONDITIONS ^(a)	FOS (% dry weight)			COLOUR A ₄₉₀
	°C (minutes)	dp=1+2	dp=3-6	dp>6
60 (80)		14.5	56.7	28.8
80 (50)		20.8	54.5	24.7
100 (20)		28.4	50.4	21.2
Crude JA flour ^(b)		33.3	46.4	20.3

- (a) 100 g batches of sliced JA tubers (0.25 cm thick) were placed in an oven set at 60, 80, and 100°C until dried. Duplicate samples from each batch (which were ground, suspended in water (0.5 g/10 mL water) and filtered) were quantitated for colour (A₄₉₀) (flour colour determined similarly) and then analyzed by HPLC to determine FOS composition. FOS content was averaged from HPLC analysis of two lots of tubers dried at each temperature. Colour was analyzed in triplicate for each batch (mean $\pm$ standard deviation). Mean moisture content of the dried slices was calculated by comparing wet and dry weight of the 100 g batches of sliced tubers was ca. 10 ± 3.0 % for all the dried JA slices.
- (b) Composition of the crude flour is based on the HPLC analysis performed in Chapter one; Fig 1.1.

commercially important fungi. Therefore I compared growth of Aspergillus niger (high citrate producer), and Penicillium chrysogenum (high penicillin producer) in a crude JA flour solution with that of growth in a standard synthetic medium containing glucose and peptone known to support good growth of fungi (Ingold, 1967).

Fungi were grown in 5 % (w/v) of JA crude flour broth because the dry matter constituents of the synthetic broth contained 4 % (w/v) glucose and 1 % (w/v) peptone - a total of 5 % (dry matter). Growth was followed by determining the increase in dry weight of mycelia until the fungi reached stationary phase. Dry weight of mycelia was determined by passing cultures (grown at 30°C in 250 mL flasks; 50 mL broth/flask) through a pre-weighed and pre-dried filter paper at appropriate time intervals. The mycelia collected were then oven-dried (at 100°C) and dry weight (g) calculated by subtracting the filter weight (g). Growth curves were constructed by plotting dry weight of mycelia (g) (on a log scale) vs. time (Fig 2.1).

Fig 2.1 shows typical growth curves (based on increase in mycelial weight) for fungi grown in the Sabour's and JA flour broths. Both fungi grew with no supplements in the JA flour broth but at a slower rate than in Sabour's medium. In addition, fungi grown in the JA flour broth had accumulated considerably less mycelial mass than in Sabour's medium. The difference in growth rates of the fungi in the JA and Sabour's may be due to the fact that the

Fig 2.1. Typical growth curves of Aspergillus niger (a) and Penicillium chrysogenum (b) in Sabour's broth and the JA crude flour broth. Four flasks containing 50 mL of each broth were inoculated with 0.5 mL of a spore suspension containing ca. 1 mg dry weight of spores of each fungi which had been freshly sporulated on Sabour's agar. The cultures were incubated at 30°C with shaking at speed #5. At different time intervals, one flask from each set was filtered through a filter paper. The mycelia collected on the filter paper was washed, dried and weighed. Growth curves were constructed by plotting dry weight of mycelia (g) on a logarithmic scale vs. time, until fungi reached stationary phase.

(a)

(b)

Sabour's broth is largely protein-based and that the JA broth is largely carbohydrate. Although protein-based media would be suitable for production of protein products (e.g. protease) the carbohydrate-based JA flour broth may provide a relatively cheap growth medium for the production of carbon-based products (e.g. citric acid). However, further experimentation is necessary to optimize conditions for the production of such products using JA products. This preliminary investigation shows that there is potential for the crude JA flour to be used as a fungal growth medium.

Materials and Methods

Materials

Yorkshire piglets, the automatic feeding systems and milk replacer ingredients used in feeding trials were supplied by Animal Research Centre branch of Agriculture Canada.

Fungi (Aspergillus niger (ATCC 11414) and Penicillium chrysogenum (ATCC 408017) (obtained from the National Research Council Laboratories, 100 Sussex Drive, Ottawa.) are both x-ray induced mutants which overproduce citric acid and penicillin, respectively.

Preparation of Sabour's agar and JA crude flour broth for growth of fungi

100 mL of a 40 % (w/v) filter sterilized glucose solution was added to 900 mL of autoclaved (20 min at 120°C) solution containing peptone (10 % (w/v) and Bacto agar (25 % w/v). Sabour's broth was prepared similarly, without agar. The JA flour broth was prepared by suspending 50 g of the flour. In 1 L of (5 % (w/v) distilled water, filtering through cheesecloth and then autoclaving at 120°C for 20 min.

Methods

Piglet Feeding Trials

Feeding system used for piglet feeding trials (Fig 2.2).

Oligosaccharide analysis of the dried JA slices

All oligosaccharide analysis was accomplished by HPLC as described in Chapter one; Methods.

Quantitation colour of the dried ground JA slices

Colour was quantitated at A₄₉₀; since the JA extract described in Chapter three showed maximum absorption at this wavelength.

Fig 2.2. Six-cage automatic feeding system for neonatal piglets. The equipment shown consists of six heated cages suspended in a mobile rack, a programmable liquid feeding system with electronic controls, as well as facilities for watering, and the collection of feces. Each unit allowed individually controlled feeding of a liquid diet to six pigs at regular time intervals. Three units housed in the same room allowed simultaneous use of the different dietary treatments (+IG, +JA, and Control). Pans under each cage allowed for the collection of feces. The cages (600 X 400 X 300 mm high) are made of 0.8 mm sheet stainless steel. Each has a removable nylon-coated screen floor and a hinged door of the same material. Localized heating to create a suitable microenvironment for the piglet is provided at the rear of the cages.

The liquid diet is circulated continuously in a closed loop from the reservoir by a metering pump (Model No. RP2ID, Fluid Meeting Inc.). To prevent deterioration in the absence of refrigeration, the milk replacer has 0.1 % of formalin (40 % formaldehyde) added as a preservative (Reid et al., 1971).

CHAPTER THREE

PRODUCTION OF PURIFIED JA FLOUR DEVOID OF COLOUR AND OFF- FLAVOUR

Introduction

The crude JA flour produced in the pilot studies (Chapter one) would not appeal to humans since it had colour and off-flavour which may have been produced by enzymic browning reactions during tuber maceration (Chapter one; Fig 1.1). In this Chapter, we attempt to develop a process to prevent or remove the undesirable colour and off-flavour during the preparation of the JA flour.

Enzymic browning occurs because the JA tubers contain an abundance of phenolic compounds (mainly chlorogenic, caffeic and ferulic acids) (Paupardin, 1965). The endogenous diphenoloxidase (DPO) can catalyze the oxidation of the phenols to highly reactive o-quinones that can react with amino acids and proteins generating complex brown polymers associated with colour and off-flavours (Walker, 1977), as shown in Fig 3.1. The formation of o-quinones is reversible and therefore reducing agents such as ascorbic acid or sodium metabisulphite can prevent enzymic browning by reducing the colourless o-quinones back to their parent phenols but when these compounds are consumed, the o-quinones are no longer reduced and undergo oxidative polymerization, yielding the brown pigments. Although ascorbic acid (vitamin C) is still one of the safest methods for the commercial control of enzymic browning, there is still opportunity for pigments to form and it can corrode equipment (Mathew and Parpia, 1979).

In this chapter, we describe the development of preparing colourless bland JA flour by employing heat inactivation of DPO and several adsorbents to remove

Fig 3.1. Generalized reaction scheme for the action of diphenoloxidases (DPO).

phenols, colour and off-flavour. Anion or cation exchange resins are routinely employed to adsorb compounds according to ionic charge. Dowex-66 is a weakly basic anion exchange resin which should adsorb phenols in solution since they are anions when deprotonated. In addition, cross-linked polyvinylpyrrolidone (PVP) may adsorb phenols because of its capacity to form strong hydrogen bonds with the hydroxyl groups of phenolic compounds (Ollson and Samuelson, 1974). Activated carbon has a high adsorptive capacity for coloured substances because of the tremendous surface area (600-1500 square meters/gram) contained in the micropore structure of the carbon particles (Norit Company Bulletin, 1986). Since activated charcoals are routinely used for production of crystalline sugar from sugar cane, they may be a suitable adsorbent for producing the purified JA flour.

The decolourizing efficiency of these adsorbents will be evaluated in terms of the minimal amount and exposure time required to produce a sufficiently decolourized standard JA extract with known colour and FOS composition which could then be dried (lyophilized) to produce suitable JA flour (lacking colour and off-flavour). In addition, the ease at which a suitable extract can be produced using the appropriate adsorbent will be taken into consideration, since the method we develop should be appropriate for a larger scale investigation (e.g. producing purified JA flour from whole JA tubers using the flour production process described in Chapter one).

Results and Discussion

In order to compare the decolourizing capacities of the different test adsorbents, it was necessary to study the extraction of FOS from the JA tubers with water. 1 kg batches of sliced JA tubers (0.25 cm thick) were extracted in different amounts of unheated water (0.5-1.25 L) to determine the optimal water to tuber ratio that would produce a JA extract with the best soluble FOS yield. The water and sliced tuber mixtures were autoclaved (120°C) for 10 and 20 min (which may denature DPO and therefore minimize discolouration) and the JA juice extracted by hand pressing the JA pulp through two layers of cheesecloth. To determine the effect of heating and the ratio of water to tuber, on the yield of total soluble sugar (g/L), the extracts were analyzed for total carbohydrate using the anthrone method (Umbreit *et al.*, 1972). Table 3.1 shows that the greatest of total soluble sugar yield (227 ± 5 g/L) was achieved when the sliced tubers were extracted in an equal volume of water which was autoclaved (120°C) for 20 min. To determine the effect of heating on colour and FOS composition; 10 mL of the heated (autoclaved for 10 or 20 min) and the unheated (control) tuber extracts (both extracted in 1 part water) were diluted 5X, filtered and then analyzed for colour at A_{490} (since the absorption of colour was maximum at this wavelength) and for FOS composition by HPLC. Table 3.2 shows that heating (120°C) for 20 min produced an extract with the least amount of colour (A_{490} ; ca. = 0.22). Although heating for 20 min

Table 3.1. The effect of water extraction and heating on the total soluble sugar yield of the JA extract^(a).

RATIO OF WATER : TUBER SLICES (L) (kg)		TOTAL SOLUBLE SUGAR (g/L)		
		DURATION OF HEATING (MINUTES)		
		0	10	20
0.5 : 1			171 $\pm$ 4	188 $\pm$ 2
0.75 : 1			188 $\pm$ 2	200 $\pm$ 6
1.0 : 1	182 $\pm$ 5 ^(b)		197 $\pm$ 7	227 $\pm$ 5
1.25 : 1			196 $\pm$ 4	214 $\pm$ 4

- (a) JA slices immersed in varying amounts of unheated water were autoclaved (120°C) for 10 and 20 min and the JA juice extracted by squeezing the pulp through two layers of cheesecloth. Samples of the extracts were then diluted 1:10 000 and mean total soluble sugar (g/L) $\pm$ standard deviation determined (based on triplicate samples), using the anthrone method and a standard curve of inulin (Fig 3.3; Methods).
- (b) Once it was determined that a 1:1 ratio of tuber : water gave optimal extraction of soluble sugars, an unheated extract was prepared and total soluble sugar determined similarly (a).

Table 3.2. The effect of heating on the FOS composition and colour of the JA extract. (a)

DURATION OF HEATING (MINUTES)	FOS (% dry weight)			COLOUR (A_{490})
	dp=1+2	dp=3-6	dp>6	
0	1.8	61.6	35.6	.37 $\pm$ 0.04
10	19.9	54.6	23.5	.30 $\pm$ 0.03
20	24.4	49.5	23.1	.22 $\pm$ 0.05

- (a) Samples of the 1:1 extracts from Table 3.1 (no heat, 10 and 20 min autoclaved (120°C)) were analyzed by HPLC (in duplicate) and average FOS content calculated for each fraction. Colour of the 5X diluted JA extract was quantitated at A_{490} and the mean colour $\pm$ standard deviation, calculated from triplicate samples.

reduced the dp 3-6 FOS and increased the content of mono- and di-saccharides, there was still a sufficient amount of these FOS (ca. 50 % dp 3-6). Therefore, this extraction process was used throughout to produce the standard JA extract which was used to compare the efficiency of the decolourizing treatments.

The decolourizing efficiency of the Dowex-66 (weakly basic anion exchanger) and PVP (PolyClar AT) was determined by exposing a fixed amount of each agent to the JA extract for increasing contact times and by comparing the colour (A_{490}) of the treated extracts to that of the untreated standard JA extract (control). Before use, two 5 g samples of the Dowex-66 resin were thoroughly washed; one with saturated sodium bicarbonate and the other with 1 N acetic acid to replace the hydroxyl group (OH^-) group on the resin with carbonate (CO_3^-) and acetate (CH_3COO^-) ions. Since these ions (CO_3^- and CH_3COO^-) are more weakly bound (Khym, 1974), they should be more easily exchanged with the acidic phenols in the JA extract which may prevent further browning reactions and reduce discolouration. Table 3.3 shows that Dowex-66 was an inefficient decolourizer of the JA extract; which was only ca. 23 % decolourized after 15 min of contact. Moreover, the pH of the JA extracts (ca. pH 6.0) was drastically lowered upon the resin treatment (e.g. ca. pH: 3.8). The low pH is undesirable since FOS hydrolysis would occur during flour preparation. However, treatment with PVP (PolyClar AT) was more efficient since the JA extract was 40 % decolourized after 15 min (with no appreciable change in pH). However, it was clear that large amounts of PVP and

Table 3.3. The effect of Dowex-66 resin and PVP treatment on the colour of the JA extract.

CONTACT TIME (MINUTES)	% COLOUR REMOVED		
	DOWEX-66 CO ₃ ⁻	CH ₃ COO ⁻	POLYCLAR AT (PVP)
2	10 ± 5	12 ± 2	23 ± 4
5	15 ± 3	19 ± 3	28 ± 2
10	20 ± 2	23 ± 2	33 ± 3
15	22 ± 2	24 ± 1	40 ± 3

- (a) Minimal amounts of each agent were employed (5 g of Dowex-66 in acetate and carbonate forms and 1.0 g of PVP to decolourize 25 mL aliquots of the 5X diluted JA extract. Resin-treated extracts were passed through a glass scintered funnel to recover the resin and then quantitated for colour at A_{490} since the extract showed the greatest absorption at this wavelength. PVP was removed by centrifugation at 5 000 rpm for 5 min. Mean % of colour removed $\pm$ standard deviation was calculated from duplicate samples of the extract from two experimental trials. % colour removed was calculated as follows at A_{490} :

$$\frac{A_{490} \text{ of the untreated JA extract} - A_{490} \text{ of the treated JA extract}}{A_{490} \text{ of the untreated JA extract}} \times 100$$

longer contact time (which would be both costly and inefficient) would be required to produce a suitably decolourized JA extract (e.g. an extract that was ca. 90 % decolourized). Thus our attention was then focused on activated charcoals (powdered and granular) as a potential decolourizing agent since small amounts of activated carbon have a large adsorptive surface area which may adsorb significant amounts of the colour and impurities produced by the browning reactions.

There are a great variety of decolourizing activated charcoals. The powdered activated charcoals we chose (Norit SA4, Darco S51 and Darco KB powdered charcoals) for decolourizing the JA extract should be suitable since they have been routinely used for water treatment, improvement of the smell and taste of beverages (e.g. wines and fruit juices), and for decolourizing vegetable oils and fats. The granular charcoals employed; Sigma (4-14 mesh), Norit ROX (12-40 mesh) and Sigma (14-60 mesh) have been used for removal of taste, odour and dissolved organics in water treatment systems (Norit Company Bulletin, 1981 and ICI America Bulletin, 1989).

The decolourizing efficiency of each of the six activated carbons (three powdered and three granular) was compared by using a limited amount of charcoal and short contact time. 25 mL of the extract was exposed to 0.2 g of the powdered charcoals for 2 min. For the granular charcoals, 25 mL of the extract was passed through a 0.5 X 10 cm mini-column bed of the granular charcoals and minimum

contact. Contact time for the granular charcoals varied between 8-10 minutes depending on the flow rate of the extract through the column. Table 3.4 shows that the Darco KB powdered activated charcoal was the most efficient decolourizer of the JA extract since 48 % of the colour in the extract was removed with minimal amount and exposure. This is not surprising since it was the powdered charcoal with the greatest adsorptive surface area (ca. 1200 m²/g; Table 3.9; Methods). Similarly, the Sigma (14-60 mesh) and the Norit ROX (12-40 mesh) granular charcoals which had the largest internal adsorptive surface area (ca. 1000-1200 m²/g) among the granular charcoals employed, were effective decolourizing granular charcoals since ca. 35 % of the colour in the JA extract was removed upon treatment. Treatment of the JA extract with activated carbon (powdered or granular) did not appreciably affect pH of the extract (ca. pH; 6.0). The results show that more charcoal or longer contact would be required to produce a more highly decolourized extract. To determine the effect of increasing the amount of charcoal on the amount of colour removed from the JA extract, the amount of Darco KB was increased from 0.2-1.2 g/25 mL of extract, keeping contact time constant at 2 min. Table 3.5 shows that addition of 0.8 g of the Darco KB powdered charcoal/25 mL of the JA extract enhanced colour removal by 12 %, producing an extract which was 60 % decolourized. However, addition of more than 0.8 g charcoal to the extract did not seem to significantly increase the amount of colour removed. Therefore using 0.8 g of Darco KB/25 mL of the extract; contact time was then increased up to 25 min to determine if decolourization could be enhanced with increased contact. Table 3.6

Table 3.4. The effect of activated charcoal treatment on the colour of the JA extract (a)

DECOLOURIZING CHARCOAL	% COLOUR REMOVED
Sigma (4-14)**	28 $\pm$ 5
Norit ROX 0.8(12-40) **	35 $\pm$ 4
Norit SA4*	35 $\pm$ 3
Sigma (14-60)**	36 $\pm$ 4
Darco S51*	37 $\pm$ 3
Darco KB*	48 $\pm$ 2

(a) Decolourizing efficiency is based on the minimal amount of powdered charcoal (0.2 g/25 mL of the JA extract (5X diluted) with minimum contact (2 min) and granular charcoals (25 mL of the extracts were passed through 0.5 X 10 cm mini-columns and contact time varied between 8-10 min, depending on the flow rate. The mean amount ($\pm$ standard deviation) of colour removed from the filtered JA extracts, were calculated from duplicate samples from two experimental trials.

* powdered charcoals; ** granular charcoal (numbers in brackets refer to mesh sizes (carbon particle sizes).

Table 3.5. The effect of increasing the amount of activated charcoal (Darco KB) on the colour of the JA extract. (a)

AMOUNT OF CHARCOAL ADDED (g)	% COLOUR REMOVED
0.2	45 $\pm$ 2
0.4	47 $\pm$ 1
0.6	56 $\pm$ 2
0.8	60 $\pm$ 3
1.0	61 $\pm$ 1
1.2	62 $\pm$ 3

- (a) The amount of activated charcoal used to treat the JA extract was increased by increments on 0.2 g from 0.2 - 1.2 g/25 mL of the JA extract; and the mean % of colour removed from the JA extract was determined from duplicate filtered samples of the treated extracts quantitated at A_{490} from two experimental trials.

Table 3.6. The effect of increasing contact time of the powdered activated charcoal on the colour of the JA extract. (a)

CONTACT TIME (MINUTES)	% COLOUR REMOVED
1.0	46 $\pm$ 3
2.0	57 $\pm$ 2
5.0	62 $\pm$ 2
10.0	67 $\pm$ 3
15.0	69 $\pm$ 2
20.0	70 $\pm$ 1
25.0	71 $\pm$ 1

- (a) 0.8 g Darco KB /25 mL of the JA extract was exposed to the extract for different contact times, filtered and % colour removed determined at A_{490} trials, and mean % colour removed calculated from duplicate samples from two experimental trials.

shows that when 0.8 g of the charcoal was exposed to the JA extract for 15 min, decolourization was enhanced a further 10 %, so that the JA extract was now ca. 70 % decolourized. Increased contact (e.g. greater than 15 min) did not seem to increase colour removal. After this charcoal treatment, the extract still appeared yellowish and had a detectable aroma. Colour removal did not seem to be further enhanced with increased addition of the charcoal or increased contact time did not reduce the remaining colour or aroma. Therefore it was concluded the colour, once formed, is difficult to remove in this batch process.

In the next experiment, we attempted to prevent the opportunity for colour development and to remove any colour that might form. The sliced tubers were immersed in one part of a boiling water mixture containing activated charcoal. This may facilitate inactivation of DPO and at the same time remove phenols and pigments by adsorption to the charcoal, minimizing discolouration. The mixture was then heated at 120°C for 20 min. This should further insure inactivation of DPO, provide sufficient contact time for the charcoal to adsorb any coloured impurities (Table 3.6) and provide optimal extraction of the soluble FOS (Table 3.1). The mixture was then squeezed through cheesecloth and filtered. Using this method, JA extracts were prepared from 100 g lots of sliced JA tubers (0.25 cm thick) suspended in 100 mL of water containing various amounts of the powdered activated charcoal. Table 3.7 shows that the addition of ca. 3.4 g of charcoal to 100 mL of boiling water for FOS extraction resulted in maximal removal of colour (the

Table 3.7. The effect of increasing the amount of powdered activated charcoal on the colour of JA extracts produced by immersing JA slices in a boiling water mixture. (a)

AMOUNT OF CHARCOAL ADDED (g)	% COLOUR REMOVED
2.6	38 $\pm$ 4
2.8	51 $\pm$ 3
3.0	67 $\pm$ 3
3.2	85 $\pm$ 4
3.4	90 $\pm$ 4
3.6	89 $\pm$ 2
3.8	90 $\pm$ 3

- (a) Colour of the hot JA extract without charcoal treatment was quantitated at A_{490} (ca. 0.14). Therefore the colour removal was calculated as described in Table 3.1 relative to the colour in this hot JA extract. 100 g of sliced JA tubers immersed in one part boiling water containing different amounts of the Darco KB activated charcoal and were autoclaved (120°C) for 20 min) and then filtered and analyzed for colour content (A_{490}). Mean colour removal ($\pm$ standard deviation) was calculated from four samples: duplicate samples from two experimental trials.

JA extract was ca. 90 % decolourized). The decolourized JA extract was then dried (lyophilized). The resulting JA flour lacked off-flavour and aroma as well as colour. Three lots of the purified JA flour were prepared from 100 g lots of sliced JA tubers using this procedure. Mean yield of the purified JA flour from 100 g of the sliced JA tubers was ca. 6.5 g, corresponding to ca. 40 % mean recovery of the tuber fructans. This could have been significantly improved if the cooked JA tuber slices were mechanically pressed rather than hand pressed. The first lot of the purified JA flour produced was used for composition analyses. FOS composition was determined by HPLC, total protein using the automated Kjeldahl procedure described in Chapter one, and total ash content determined using the method of Umbreit *et al.* (1972). Soluble carbohydrate content was estimated by subtracting total protein and ash from 100 since there was no insoluble component (composition of the purified JA flour is summarized in Fig 3.2). The high ash content of the flour may arise from the use of charcoal (Methods; Table 3.9) or could result from the cultivation of the tubers in clay soil which has a higher ash content than sandy soil (Dr. Jones, personal communication, 1988).

Although powdered activated charcoal proved to be the most efficient adsorbent, removal of the fine charcoal particles was difficult. However, industrial filtration equipment would be capable of filtering without contamination of fine charcoal particles. Therefore, we also examined the use of granular charcoal columns for removal of colour from the boiling water tuber extract. The hot JA

Fig 3.2 Typical FOS composition of the purified JA flour. Numbers above the peaks refer to degree of polymerization (dp). HPLC columns allowed separation of oligosaccharides through dp 9; with the longer chain FOS (dp>9) coming off the column first. Typical FOS composition of the purified flour is as follows in % dry weight in terms of degree of polymerization (dp): dp=1+2; fructose or glucose plus sucrose, ca. 34.2, FOS dp=3-6; ca. 45.2, FOS dp>6; ca. 20.6. The composition of the purified water-soluble JA flour (in % dry weight) is as follows: soluble carbohydrate, ca. 92; protein, 1.6 and total ash, 6.2.

FOS COMPOSITION (%)

dp=1	10.0
dp=2	24.2
dp=3	15.3
dp=4	12.0
dp=5	9.5
dp=6	8.4
dp=7	6.8
dp=8	4.0
dp=9	2.6
dp>9	7.2

extracts (produced without addition of charcoal), were passed through mini-columns (0.5 X 10 cm) packed with the granular charcoals to determine the extent of decolourization. Table 3.8 shows that the Sigma (14-60 mesh) and the Norit ROX (12-40 mesh) granular charcoals have the potential to produce such an extract since the hot JA extracts were ca. 70-75 % decolourized. Although decolourization was not as effective as extraction in the boiling water tuber suspension containing the powdered charcoal, granular charcoal beds may remove colour to a greater extent if larger beds were used. The decolourization procedure developed is summarized in Fig 3.3).

If the purified JA flour could be shown to be bifidogenic (i.e. stimulating growth of bifidobacteria) the purified JA preparation could be used as a food additive in human foods. In the following Chapter we describe the potential of the purified JA flour as a bifidogenic agent.

Table 3.8. The effect of granular charcoal treatment on the colour of the hot JA extract^(a).

GRANULAR CHARCOAL	% COLOUR REMOVED
Sigma (4-14) ^(b)	25
Norit ROX 0.8 (12-40)	70
Sigma (14-60)	75

- (a) Colour removal was calculated relative to the colour of the hot JA extract (A_{490} : ca. 0.14) prepared without charcoal treatment as described in Table 3.7. 25 mL aliquots of the JA extract prepared without addition of powdered charcoal were immediately passed through mini-columns (0.5 cm X 10 cm) containing the different granular charcoals (contact between 8-10 min; depending on the flow rate) and then quantitated for colour at A_{490} . Average % colour removed was calculated based on samples from two experimental trials.
- (b) Numbers in brackets refer to the mesh size of the granular charcoals.

- 1) Sliced tubers are immediately mixed with 1.0 part boiling water.
- 2) The mixtures are then cooked under pressure (we used an autoclave (120°C) for 20 min in the presence (A) or absence (B) of powdered activated charcoal (e.g. Darco KB)
- 3) The mixtures are squeezed through a cloth. For (A) the juice is filtered. For (B), the filtered juice is passed through a column of granular activated charcoal (e.g. Sigma 14-60 mesh or Norit ROX 12-40 mesh).
- 4) Spray-drying (we used lyophilization).

Products : free of colour and lacks off-flavour.

Fig 3.3. Decolourization process developed for production of the colourless purified JA flour.

Materials and Methods

Materials

Jerusalem artichoke tubers (Columbia variety) used in this study were harvested in late October 1988 and supplied by Agriculture Canada, Ottawa, Ontario. Tubers used in these studies had been stored in plastic bags and refrigerated (4°C) for several months.

The Dowex-66 weakly basic anion exchange resin was supplied by the Dow Chemical Company, Don Mills, Ontario. All Norit and Darco brand activated characoals (powdered and granular) were supplied by the American Norit Company, Jacksonville, Florida (distributed by Van Waters & Rogers, Montreal, Quebec, Canada). All other chemicals used were of analytical grade and supplied by Sigma Chemical Company.

Materials

Table 3.9. General characteristics of the activated carbons (powdered and granular) used to decolourize the JA extracts.

POWDERED CHARCOALS			
GENERAL CHARACTERISTICS	NORIT SA-4 *(A-4973)	DARCO S-51 (A-5487)	DARCO KB (A-5489)
Total surface area (m ₂ /g)	650	650	1500
Bulk density (lbs/cu.ft.)	23	32	28
Mesh size, % thru 100	89	98	99
% thru 325	77	70	70
Ash content, %	6	3	7
Moisture content, %	2	10	33
Water soluble, %	2.5	1.5	1.5
GRANULAR CHARCOALS	MESH SIZE	INTERNAL SURFACE AREA (m ² /g)	
Sigma (18F-0281)	4-14	660	
Norit ROX 0.8 (A-3397)	12-40	1000	
Sigma (C-3014)	14-60	1200	

Source: (Norit Company Bulletin, 1981)

* these are catalogue numbers

Preparation of anthrone reagent

2.0 g of anthrone (9-oxyanthracene) is dissolved in 1 L. of 95 % sulfuric acid (prepared by cautious addition of 1 L. of concentrated sulfuric acid to 50 mL. of water with cooling). The reagent darkens with time so it was prepared fresh every time in 100 mL quantities.

Methods

Total soluble sugar determination

Anthrone reacts with all carbohydrates to give a characteristic blue-green colour at A_{620} (Umbreit *et al.*, 1972). A calibration curve using inulin as the standard carbohydrate was constructed using the anthrone method (Fig 3.4). Triplicate samples of 10 $\mu\text{g/mL}$ - 80 $\mu\text{g/mL}$ inulin were assayed (since practical range of the assay is 10-100 $\mu\text{g/mL}$) (Umbreit *et al.*, 1972) as follows: 3 mL of the samples were determined in 18 X 150 mm test to which 6 mL of anthrone reagent was added. Solutions were mixed thoroughly by gently vortexing (speed #3). The tubes were then placed in a boiling water bath for 3 min and cooled to room temperature. Colour was quantitated at 620 nm on a spectrophotometer. A blank containing 6 mL of anthrone and 3 mL of distilled water was treated similarly. Samples of the JA extract were assayed and total soluble sugar determined from

Fig 3.4 Standard curve of inulin using the anthrone method
for determination of total soluble sugar (g/L) in
the JA extracts.

the standard curve of inulin as follows: ($\mu\text{g/L}$) (from standard curve) $\times$ Dilution factor = Total soluble sugar (g/L).

Filtration of the powdered charcoals

The samples of the JA extract treated with powdered activated charcoal were first filtered through a Whatman # 1 filter and then twice through a Whatman # 42. The JA extracts that were treated with granular charcoal were filtered through a Whatman # 1 to remove residual charcoal.

Quantitation of the colour in the JA extracts

An absorption spectrum of a filtered 5X diluted sample of the JA extract [1 (kg) sliced tuber (0.25 cm) : 1 (L) unheated water] showed that maximum absorption occurred at $A_{490\text{nm}}$ (ca. 0.22). Therefore this wavelength was used throughout to quantitate colour of the JA extracts. The hot JA juice (5X diluted) extracted from the JA slices immersed in boiling water A_{490} was ca. 0.14.

Determination of the % colour removed from the JA extract :

% Colour removed (or % decolourized) from the (5X diluted) JA the extract =

$$\frac{A_{490} \text{ of the untreated standard JA extract (ca. 0.22)} - A_{490} \text{ of the treated JA extract (with an adsorbent)}}{A_{490} \text{ of untreated standard JA extract (ca. 0.22)}} \times 100$$

Calculation of % yield of the purified JA flour from JA tubers

Mean yield of the purified JA flour was calculated based on 20 % dry weight

of tubers (solids) (Table 1; General Introduction). 100 g of sliced tubers (0.25 cm thick) should contain ca. 16 g of fructans (since ca. 80 % of the dry weight of the tubers are carbohydrate; Table 1). Since the average yield of the flour using the batch process described in Fig 3.3 (Procedure A) was ca. 6.5 g (from three 100 g lots of sliced tubers). Therefore $100 \times 6.5 \text{ g flour} / 16 \text{ g of fructans} = \text{ca. } 40 \%$ recovery of the fructans in the JA tubers.

Analyses of the main components of the purified JA flour

All samples purified JA FOS-rich flour were analyzed with a new set of columns TSK-Oligo (#1173) which showed greater resolution of oligosaccharides resolution than the columns used in Chapter one. The solvent system was the same as that described in Chapter one. All samples were prepared as outlined in the Methods in Chapter one. Average total protein was determined using an the automated Kjeldahl system described in Chapter one; Methods. Ash was determined using the method described by Umbreit *et al.* 1972, also described in Chapter one. Average protein and ash were determined by analyzing the purified flour in duplicate. Total soluble carbohydrate was estimated by subtracting total protein plus ash minus 100, since the purified JA flour was totally soluble in water and soluble carbohydrates are the main component in the tubers (Table 1; General Introduction).

CHAPTER FOUR

UTILIZATION OF PURIFIED JA FOS FLOUR BY BIFIDOBACTERIA AND YEASTS

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

Introduction

Bacterial species isolated from the human gut include diverse genera comprising Gram-negative and Gram-positive, anaerobic and facultatively anaerobic rods, and cocci. Some bacterial groups predominate in the intestine such as bacteroides and bifidobacteria, while others occur in only small numbers in the normal gut flora such as enterococci and enterobacteria, or are a minority; staphylococci, clostridia and aerobic spore formers (Rasic, 1983). Some of these gut organisms may have important metabolic effects and interactions with the host (Rasic, 1983). It is well established that the anaerobic bifidobacteria are beneficial intestinal bacteria in humans as well as some animals. Bifidobacteria are Gram-positive, non-sporing rods which ferment carbohydrates to acetic and lactic acids. Bifidobacterium infantis predominates in human infants whereas B. adolescentis and B. longum are dominant in adults (Mitsuoka, 1982). Accumulating evidence suggests that the metabolic activities of bifidobacteria are responsible for providing a variety of health benefits to the host (Rasic, 1983; Hidaka et al., 1986). Acetic and lactic acids produced from catabolism of carbon sources lowers intestinal pH inhibiting unfavourable bacteria such as E. coli and Clostridium perfringens which produce toxins and can cause enteritis. The acids also reduce absorption of ammonia and amines which if they are absorbed, lead to increase in blood pressure, cholesterol and levels of cancer-causing nitrosamines. Therefore, intestinal propagation of bifidobacteria is not only important for preventing bacterial diseases such as E. coli

scours in infants but for improving the health of adults, particularly the elderly. In addition, bifidobacteria provide vitamins B complex and stimulate intestinal immunity (Deguchi *et al.*, 1985; Yamazaki *et al.*, 1985).

A variety of growth-stimulating bifidogenic agents have been identified (Rasic, 1983). Since these agents are available in limited quantity in nature (e.g. in breast milk), they have been produced by enzymic or chemical modification of natural products (e.g. lactulose from lactose) (Hoffman, 1976). FOS, chemically derived from inulin, have been shown to stimulate growth of bifidobacteria (Yazawa and Tamura, 1982). Thus, an FOS preparation known as Meioligo P (enzymically derived from sucrose) has been commercialized in Japan because it reduces constipation, blood pressure, blood lipids and cholesterol in humans; it reduces scours and increases weight gain and feed efficiency in piglets (Hidaka *et al.*, 1986).

There is an increasing consumer demand for natural products particularly for food or food additives. Among inulin producing plants, the JA produces the highest amount of inulofructosaccharides in its tubers. In Chapter three we described a process for producing purified JA flour from JA tubers. In this Chapter we wish to examine how well this purified JA flour preparation (which contains a high proportion of FOS) will support the growth of bifidobacteria.

Most studies on bifidogenic agents have used the extent of growth (cell density after reaching stationary growth) as a measure of growth stimulating capacity. However, it is the rate of growth that compares the efficacy of these bifidogenic agents. The present communication describes a simple method that

obviates the use of an anaerobic chamber for continuous measurement of growth of bifidobacteria in presence of inulofructosaccharides of various FOS compositions.

If the purified flour is proved to be bifidogenic, then the next logical application would be to add it into human food. Addition in leavened products, particularly bread, would be an ideal North American application of our product since bread is consumed daily. Since the *B*-fructosidase of bread yeast efficiently hydrolyzes small-sized FOS (e.g. GF₁-GF₆); major components of the Japanese derived FOS, the dietary value of the FOS may be lost. However, our purified JA flour contains large-sized FOS (ca. 25 % dry weight) which may be hydrolyzed more slowly by *B*-fructosidase, generating small-sized FOS during bread-rising. It is also possible that yeast will be able to utilize all the FOS contained in our product. Thus in this Chapter the utilization of the JA FOS preparation by yeast is also studied.

Results and Discussion

Measurement of the growth of bifidobacteria on inulofructosaccharides

Although an anaerobic chamber is commonly used to grow bifidobacteria, it is inconvenient for continuous measurement of growth. Here we have found that bifidobacteria can grow well under appropriate air evacuation. We placed TPY broth (containing 1 % (w/v) fructose) in culture tubes that could be fitted into a spectrophotometer to study the effect of evacuation on growth of *B. infantis*. Table

4.1 shows that two to three minutes of evacuation (100 mm Hg) provided optimal conditions for growth of the *B. infantis*. Similarly, 2.5 min evacuation gave optimal growth for *B. adolescentis* and *B. longum* (Table 4.2). Therefore, 2.5 min evacuation was used throughout.

To allow the measurement of both extent and rate of culture growth on different carbon sources, TPY medium was supplemented with limited amounts (0.1 % (w/v)) of the carbon sources so that stationary growth results from carbon source depletion. Growth on inulofructosaccharides (Meiologo P, crude JA flour, purified JA flour and D inulin) was compared to growth on fructose as a control (Fig 4.1). Fig 4.1 parts a,b,c show typical growth curves on these carbon sources. The figures clearly show that carbon sources were important for stimulating growth of bifidobacteria since only minimal growth was apparent on TPY broth without any carbohydrate (though rich in peptides). Although fructose provided fastest growth, fructose (also glucose and sucrose) would be utilized by humans and thus would not be available to bifidobacteria which predominate in the lower intestine (Mitsuoka, 1982). *In vivo*, it is the unutilizable carbon sources such as fructosaccharides that would be available to bifidobacteria. Both purified JA flour and Meiologo P supported good growth of all three bifidobacteria tested. Growth on the crude JA

Table 4.1. The effect of evacuation time on growth rate of B. infantis in TPY broth containing 0.1 % (w/v) fructose^(a).

EVACUATION TIME (MINUTES)	MEAN DOUBLING TIME (MINUTES)
0	184 $\pm$ 7
1	138 $\pm$ 4
2	131 $\pm$ 3
3	132 $\pm$ 4
4	135 $\pm$ 3
5	152 $\pm$ 5
10	215 $\pm$ 7

- (a) Seven culture tubes (18 X 150 mm) fitted with a two-way stopcock (0.5 cm bore size) were inoculated with 0.5 mL of a suspension of B. infantis freshly grown on TPY agar (0.5 % glucose). Six of the tubes were then evacuated using a water aspirator (100 mm Hg) for different times (1-10 min) and then incubated (without shaking) at 37°C. The control tube was not evacuated. Growth was measured at A_{510} , by inserting the tubes directly into the spectrophotometer. Mean doubling times were calculated by measuring the growth of B. infantis under these conditions three times.

Table 4.2. The effect of evacuation time on the growth rates of B. adolescentis and B. longum in TPY broth containing 0.1 % fructose^(a).

EVACUATION TIME (MINUTES)		
	<u>B. adolescentis</u>	<u>B. longum</u>
0	150 $\pm$ 5	212 $\pm$ 7
2	126 $\pm$ 3	130 $\pm$ 5
5	135 $\pm$ 4	135 $\pm$ 5

- (a) The growth study described in Table 4.1 was repeated using B. adolescentis and B. longum which were evacuated for 2 and 5 min. Doubling times were calculated as described in Methods and mean doubling times were calculated from three trials of this experiment for each bacteria.

Fig 4.1. Typical growth curves of Bifidobacterium infantis (a), adolescentis (b), and longum (c), in TPY broth with 0.1 % fructose, Meioligo P, purified JA flour, crude JA flour and D inulin.

flour (dried homogenate of the JA tubers) was slower but faster than on dahlia inulin. *B. longum* utilized inulin markedly slower (Fig 4.1 c) than *B. infantis* and *B. adolescentis* indicating that it may be deficient in the enzymes for hydrolyzing the high molecular weight FOS. In all other growth curves, the cell densities (A_{510}) on all substrates eventually reached similar final stationary levels (extent of growth) after 500 to 600 min. Thus efficacy of these carbon sources can only be assessed from the doubling times. Growth on these carbon sources was measured four times and mean doubling times are shown in Table 4.3.

FOS extracted from the JA tubers provided growth as good as the enzymically synthesized FOS (Meiologo F). Natural products (purified and crude JA flour) obtained from a long tested human food would be attractive to consumers and regulatory agencies. Since these natural products contained mostly shorter chain FOS (dp 3-6; 48 % dry weight of FOS in the tubers) which are highly soluble in water and utilized by humans, these products also have commercial potential as soluble dietary fiber. A simple method for measuring growth of bifidobacteria without use of an anaerobic chamber would facilitate future studies on bifidogenic agents.

Utilization of the purified JA flour by yeasts

To determine the fate of FOS during bread-making, four small loaves of bread dough were prepared using 100 g of white flour supplemented with 2.5 g of

Table 4.3. Mean doubling times of B. infantis, B. adolescentis and B. longum grown on TPY broth with 0.1 % of the fructose-containing carbohydrate sources (a).

CARBON SOURCE (0.1 %)	MEAN DOUBLING TIME (MINUTES)		
	<u>B. infantis</u>	<u>B.ado-</u> <u>lescentis</u>	<u>B. longum</u>
Fructose	128 $\pm$ 3	130 $\pm$ 4	136 $\pm$ 5
Meiologo P	141 $\pm$ 3	150 $\pm$ 4	158 $\pm$ 4
Purified JA flour	149 $\pm$ 4	159 $\pm$ 3	165 $\pm$ 6
Crude JA flour	185 $\pm$ 3	168 $\pm$ 5	180 $\pm$ 5
D Inulin	205 $\pm$ 6	207 $\pm$ 7	395 $\pm$ 6

- (a) Culture tubes (18 X 150 mm) fitted with a two-way stopcock (0.5 cm bore-size) containing 9.5 mL of TPY broth (with 0.1 % w/v each carbon source) were inoculated with a 0.5 mL suspension of bacteria freshly grown on TPY agar (0.5 % w/v glucose). All tubes were evacuated using a water aspirator (100 mm Hg) for 2.5 min then incubated (without shaking) at 37°C. Growth was followed at A_{510} and doubling times were calculated as described in Table 4.1. Mean doubling times were measured four times for each bacteria on each carbon source.

Meiologo P or purified JA flour. Before addition of yeast, FOS were extracted from one of the JA or Meiologo P-containing doughs by mincing and soaking them in water. The mixture was then filtered and then analyzed by HPLC. These samples would give the FOS compositions of the dough before rising. The two remaining loaves were inoculated with 0.1 g of the commercial Baker's yeast (suspended in 5 mL of water) and then left to rise at 25°C for 90 min. After rising, FOS were extracted from these loaves in water and filtered.

HPLC analysis of these filtrates (Fig 4.2) allowed us to determine the fate of the FOS in the bread dough after incubation with the yeast. The total area under the chromatograms is assumed to be proportional to the amount of FOS (that is, the refractive indices of each oligosaccharide are assumed to be the same). Fig 4.2 shows that the area under the chromatograms for each of the FOS-containing loaves was reduced by ca. 30 % after bread rising. This indicates that these sugars were utilized by Baker's yeast. Fig 4.2 also shows that larger size FOS disappeared with an accompanying increase in mono- and di-saccharides, indicating that yeast can breakdown these FOS (presumably by the action of *B-fructosidase*). Thus the dietary value of these FOS-containing products may be reduced during bread-making. To further confirm this, a study was carried out using a pure strain of yeast (*Saccharomyces cerevisiae*) instead of the commercial yeast preparation, which may have contained contaminating microorganisms capable of degrading FOS. Eight culture tubes containing 9.5 mL of yeast nitrogen broth containing 1 % (w/v)

Fig 4.2. Utilization of Meioligo P (a) and the purified JA flour (b) by Baker's yeast during bread-making. The loaves of bread dough were made by the addition of 2.5 g of each FOS preparation: Meioligo P or purified JA flour, to 100 g of white flour in ca. 20 mL of water. The FOS in the loaves were extracted by minicing up the small loaves, soaking them in 50 mL of water and squeezing out the extract through cheesecloth and analyzing the extract by HPLC. This was accomplished before addition of yeast (control) and after rising (for 90 min at 25°C) which is due to the yeast activity. After incubation with yeast, the FOS composition for the Meioligo P-containing dough was (in % dry weight): mono- and di-saccharides, $dp=1+2$; 90.6, FOS $dp=3-6$; 9.7, FOS $dp>6$; 0.0 and for purified JA flour-containing dough, mono- and di-saccharides, $dp=1+2$; 90.1, FOS $dp=3-6$; 9.4, FOS $dp>6$; 0.5.

(a)

MEIOLIGO P

FOS COMPOSITION (%)
BEFORE RISING

dp = 1	10.2
dp = 2	12.9
dp = 3	27.9
dp = 4	29.0
dp = 5	15.1
dp = 6	4.9
dp = 7	-
dp = 8	-
dp = 9	-
dp > 9	-

MEIOLIGO P

FOS COMPOSITION (%)
AFTER RISING

dp = 1	27.2
dp = 2	63.1
dp = 3	8.1
dp = 4	0.7
dp = 5	0.9
dp = 6	-
dp = 7	-
dp = 8	-
dp = 9	-
dp > 9	-

(b)

PURIFIED JA FLOUR

FOS COMPOSITION (%)
BEFORE RISING

dp = 1	9.9
dp = 2	23.2
dp = 3	14.2
dp = 4	12.0
dp = 5	9.9
dp = 6	7.9
dp = 7	6.2
dp = 8	4.0
dp = 9	3.0
dp > 9	9.7

PURIFIED JA FLOUR

FOS COMPOSITION (%)
AFTER RISING

dp = 1	26.8
dp = 2	63.7
dp = 3	4.8
dp = 4	3.6
dp = 5	1.1
dp = 6	-
dp = 7	-
dp = 8	-
dp = 9	-
dp > 9	-

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Fig 4.3. Utilization of the purified JA flour and Meiologo P by Saccharomyces cerevisiae. Cultures of S. cerevisiae grown in yeast nitrogen base containing 1 % (w/v) of each carbon source (Meiologo P and purified JA flour) were inoculated with 0.5 mL of a S. cerevisiae suspension grown to 0.6 at 30°C (O.D. 500 nm) in yeast nitrogen base containing 1 % (w/v) fructose. Shown here are the HPLC chromatograms of the filtrates extracted from the yeast cultures grown for 0 (control), 2, 4, and 6 hours at 30°C (with shaking at speed # 6).

TIME (HOURS)

0

2

4

6

(a)

% Total area (100)

% Total area (22)

% Total area (16)

% Total area (8)

PURIFIED JA FLOUR
FOS COMPOSITION (%)

TIME (HOURS)

	0	2	4	6
dp = 1	9.1	23.2	33.8	41.6
dp = 2	22.5	38.6	38.2	11.5
dp = 3	12.9	18.2	28.0	46.9
dp = 4	13.4	-	-	-
dp = 5	10.1	-	-	-
dp = 6	6.2	-	-	-
dp = 7	6.9	-	-	-
dp = 8	4.5	-	-	-
dp = 9	4.2	-	-	-
dp > 9	10.2	-	-	-

(b)

% Total area (100)

% Total area (67)

% Total area (34)

% Total area (19)

MEIOLIGO P
FOS COMPOSITION (%)

TIME (HOURS)

	0	2	4	6
dp = 1	10.7	11.5	18.0	29.7
dp = 2	13.5	54.4	47.4	26.7
dp = 3	27.1	28.6	30.3	28.9
dp = 4	29.2	-	-	-
dp = 5	16.7	5.5	4.3	14.7
dp = 6	2.8	-	-	-
dp = 7	-	-	-	-
dp = 8	-	-	-	-
dp = 9	-	-	-	-
dp > 9	-	-	-	-

yeast would have to be supplemented with glucose or fructose to generate the necessary gassing power which is essential for bread-rising.

Materials and Methods

Materials

Meiologo P was supplied by Meiji Seika Kaisha Ltd. of Japan. Bifidobacteria strains (Bifidobacterium infantis (ATCC 15697), Bifidobacterium adolescentis (ATCC 15703), and Bifidobacterium longum (ATCC 15707) and their growth medium were supplied by the Food Research Centre branch of Agriculture Canada. All other media ingredients were obtained from Difco Laboratories, Detroit, Michigan, U.S.A.

Saccharomyces cerevisiae (#X2180-1B)(Yeast Genetic Stock Centre) was obtained from the National Research Council. Fresh Baker's yeast was kindly provided by Fleischmann Co., Montreal, Canada.

Carbohydrate sources

The inulofructosaccharides used were analyzed by HPLC (Aitzetmuller, 1978) using two TSK-Oligo columns (as described in Chapter one and three). Meiologo P (Meiji Seika Kaisha Ltd.) as the standard. Oligosaccharide analyses of the inulofructosaccharides used in the growth studies of the bifidobacteria are shown in Table 4.4.

Table 4.4. FOS compositions of the fructose-containing carbon sources used for the growth of bifidobacteria.^(a)

	% dry weight of FOS			
	D inulin	Meiologo P	Purified JA flour	Crude JA flour
G or F	0.0	10.7	9.1	13.6
GF	0.8	15.2	22.1	19.7
GF ₂	0.5	29.3	12.9	13.9
GF ₃	1.2	29.6	13.4	12.2
GF ₄	1.5	10.8	10.1	11.9
GF ₅	0.0	4.4	6.2	8.4
GF _{>5}	96.0	0.0	26.2	20.3

- (a) Inulin was from Dahlia tubers (D inulin; Sigma I-3754). G represents glucose and F represents fructose. Composition of the JA products varies with harvest time and storage conditions of the JA tubers used.

Preparation of Yeast nitrogen broth

Yeast nitrogen broth was prepared by adding 10 mL of a filtered sterilized solution containing 10 % (w/v) carbohydrate (purified JA flour or Meioligo P) to 90 mL yeast nitrogen base solution (prepared by addition of 0.67 g of the yeast nitrogen base (Difco #0392 15-9) to 100 mL of distilled water which was autoclaved (120°C) for 20 min) and then inoculated with 0.5 mL of a yeast culture (grown to 0.6 O.D. 500 nm on yeast nitrogen broth containing 1.0 % (w/v) fructose).

Composition of and preparation of Trypticase Phytone Yeast extract growth medium (TPY broth) for bifidobacteria

	medium composition (g/L)
Trypticase	15.0
Phytone	5.0
Yeast extract	2.5
Carbohydrate	0.1
Tween-80	1.0
L-cysteine	0.5
K_2HPO_4	2.0
$MgCl_2 \cdot 6H_2O$	0.5
$ZnSO_4 \cdot 7H_2O$	0.25
$CaCl_2$	0.15
$FeCl_3$	0.001

Source : (Scardovi, 1986).

TPY broth was prepared by adding 10 mL of a 1 % (w/v) filter sterilized solution of each carbohydrate : fructose, purified JA flour, crude JA flour, and Meioligo P and D inulin, to 90 mL of TPY medium. Bacteria freshly grown on TPY agar (1.5 % (w/v) agar and 1 % (w/v) glucose) were suspended in 10 mL of TPY medium (without carbohydrate) and 0.5 mL of this suspension was used to inoculate 10 mL aliquots of the different FOS-containing TPY broths.

Methods

Measurement of doubling time

Growth of the bifidobacteria in each tube was followed at A_{510} and doubling times were calculated from the linear region of the $\log(A_{510})$ vs. time plots as follows:

$$T(\text{db}) = \frac{(T_2 - T_1) \log 2}{\log(A_2) - \log(A_1)}$$

$T_2 - T_1$ = time elapsed between A_2 and A_1

$A_2 - A_1$ = A_{510} of two points from linear part of curve

$T(\text{db})$ = doubling time

CONCLUSIONS

I have shown the simplest method for producing both crude and purified flour from the Jerusalem artichoke tubers. This method is rapid and therefore permits the processing of large amounts of tubers; minimizing requirement for storage. The flour produced can be stored at room temperature and is easy to dispense.

In collaboration with the Animal Research Centre, we have investigated the potential of crude JA flour as an ingredient in a piglet milk replacer. The preliminary feeding trials suggest that JA may be as effective as immunoglobulins for reducing the severity of scouring for piglets which had been exposed to the sow for 18-24 hours. However, more feeding trials are necessary to establish this conclusively. I also describe a method for preparing dried JA slices which would be useful for future administration of JA FOS to older animals.

I have demonstrated that the purified JA flour which contains both small and long chain FOS can support as good growth of bifidobacteria as the enzymically produced Meioligo P preparation which contains only small-sized FOS. This natural FOS preparation (JA flour) could be added as a 'bifidogenic' agent in many human foods (e.g. such as infant formula, or in food for elderly patients). In addition, I provide a simple method for growing bifidobacteria which would facilitate further studies on 'bifidogenic' agents.

Before this investigation, no commercial process (other than fructose syrup) was proposed for the JA tubers. The JA flour products developed here will provide

commercial opportunity for the Jerusalem artichoke which would not only improve human health but also benefit the agricultural community in Canada.

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