Sensory analysis of juice blend containing isomalto-oligosaccharides produced by fermentation with *Weissella cibaria*

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Highlights

- Transglycosylation of dextransucrase from *Weissella cibaria* was observed in orange juice and malt extract blend.
- The enzyme synthesized the isomaltooligosaccharides isomaltotriose and panose.
- Blends exhibited good acceptability, with near-ideal acidity and sweetness.

ABSTRACT

This study aimed at producing isomaltooligosaccharides in juice blends using orange juice and malt extract and assessing their acceptability. Different blend formulations were prepared and fermented, varying the concentration of orange juice, sucrose and malt extract. Dextransucrase from *Weissella cibaria* 10M was used to enzymatically synthesize α(1-6) linked glucan-oligosaccharides by transglycosylation reactions, with maltose as acceptor carbohydrate and sucrose as donor. The optimal yield of oligosaccharides was after 24 h, producing 19.4 g/L of oligosaccharides (degree of polymerization 3) from 36 g/L maltose and 19 g/L sucrose. All the blend proved to be good alternatives for synthesizing isomalto-oligosaccharides with different degrees of polymerization. Sensory analysis showed good average acceptability compared to natural orange juice, achieving scores of around 6 on a 9-point hedonic scale. In a comprehensive analysis, juice blends containing orange juice and malt extract with *Weissella cibaria* to produce oligosaccharides exhibited good sensory indicators as an innovative prebiotic beverage. A prebiotic oligosaccharide beverage can be produced by enzymatic synthesis of oligosaccharides with different degrees of polymerization.

Keywords: prebiotic, functional beverage, *Weissella cibaria*, malt, transglycosylation, oligosaccharides, sensory analysis.

1. INTRODUCTION

Fruit juices are considered excellent sources of energy, nutrients and fiber, and are consumed by all age groups. In addition, juice can be used as a vehicle for functional bioactive compounds that promote health benefits for consumers. Fruit juices contain varying amounts of sucrose and glucose. The juice of fruits with high sugar content contains correspondingly high levels of sugar (Smith and Davis, 1995). Consuming large amounts of high-sugar fruit and fruit juices may not be healthy for individuals , especially as it relates to the development of *Diabetes mellitus* (Serpen, 2012).

Diabetes, a major public health problem, is considered one of the most important non-communicable diseases in the world (WHO, 2016). Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. This reflects an increase in associated risk factors such as being overweight or obese, and 1.5 million deaths were reported in 2012. Higher-than-optimal blood glucose caused an additional 2.2 million deaths by increasing the risks of cardiovascular and other diseases. The food industry aims to develop new products with low sugar content and rich in healthy carbohydrates such as prebiotics.

Fermentation with lactic acid bacteria allows conversion of sucrose to prebiotic oligosaccharides, which occurs in many fermented foods including fermented fruits (Pedreschi et al., 2003; Stanton et al., 2008; Rabelo et al., 2009; Vergara et al., 2010; Marco et al., 2017). Fermented foods can also exhibit enhanced nutritional and functional properties due to the transformation of substrates and formation of bioactive or bioavailable end-products, and the presence of viable microorganisms (Marco et al., 2017).

Leuconostoc and *Weissella* produce extracellular oligosaccharides and polysaccharides by glucansucrases (Leemhuis et al., 2013). Dextransucrase uses sucrose as substrate to catalyse sucrose hydrolysis or dextran synthesis (Leemhuis et al., 2013; Chen et al., 2016; Hu et al., 2017). This enzyme catalyzes the formation of dextran in mediums containing sucrose as a single substrate. During synthesis, dextran remains bound to the glucan binding domain of dextransucrases (Ebert and Schenk, 1968; Heincke, 1999). Dextran has a relative molecular mass of $10^6 – 10^9$ and is used in the food industry as a gelling agent, thickener and food stabilizer. When an acceptor is present in the culture medium, oligosaccharides are produced at the expense of dextran synthesis (Leemhuis et al., 2013; Galle et al., 2010; Hu et al., 2017). Dextransucrase uses maltose, isomaltose, and glucose as acceptor carbohydrates to generate oligosaccharides (Robyt and Eklund, 1983, Hu et al., 2017). Dextransucrase thus allows synthesis of isomalto-oligosaccharides (IMO) by its acceptor reaction with maltose and glucose (Robyt and Eklund, 1983; Kothari and Goyal, 2013). An alternative way of IMO production involves the hydrolysis of starch into dextrins using α- amylase (E.C. 3.2.1.1) and β- amylase (E.C. 3.2.1.2) and then conversion to α-(1→6)- linked oligosaccharides using α-D-glucosidase (E.C. 3.2.1.20) (Delattre and Vijayalaksmi, 2000).

The dextran-producing *Weissella* spp. has received considerable attention in recent years due to its ability to produce high quantities of dextran from sucrose (Galle et al., 2010, Katina et al., 2009). Dextran produced by *Weissella* spp. consists of predominantly α-(1→6) linkages and only a few α-(1→3) branch linkages (2.4–4%), with some elongated branches (Bejar et al., 2013, Maina et al., 2011). Dextran-producing *Leuconostoc* spp. have been used to produce dextran and isomalto-oligosaccharides in wort-based beverages, but dextransucrases from *Weissella* spp.,

especially *W. cibaria*, have not been studied using fermented orange juice and malt extract blends.

IMO with α(1→6) and α(1→4) glucosidic linkages are used as low-calorie sweeteners in food products (Goffin et al., 2011). Commercial IMO are predominantly obtained from fungal glycosyltransferases using maltodextrins as feedstock, alternative methods of production use transglucosylation of maltose with dextransucrase and sucrose as glucosyl-donor (Seibel and Buchholz, 2010).

The synthesis of oligosaccharides with prebiotic properties can be carried out using the sugars present in orange juice. Therefore, the aim of this paper was to develop a prebiotic orange juice blend containing isomaltooligosaccharides from lactic acid bacteria fermentation. In the present study, dextransucrase from the dextran producer *W.cibaria* 10M was used to produce oligosaccharides by acceptor reaction with malt extract and concentrated orange juice.

2. MATERIAL AND METHODS

2.1 Material

Commercial orange juice concentrate, sucrose and baking soda were purchased from a supermarket in Edmonton, Canada. Commercial malt extract was obtained from Briess® (Briess Malt & Ingredients Co, Chilton, Wisconsin, USA). Other chemicals were purchased from Sigma Aldrich (Sigma, Oakville, Canada).

2.2 Microorganism and culture conditions

Weissella cibaria 10M was streaked on modified MRS agar plate from stock culture stored at -80 ^{o}C. Single colonies were incubated anaerobically at 30 ^{o}C (Schwab, et al. 2008, Galle et al. 2010). mMRS broth was prepared by filter-sterilized

sugar solutions to produce autoclaved MRS broth. To prepare working cultures, single colonies were removed from the agar plate and subcultured twice for 16h in MRS broth containing 24 mM maltose, 22 mM glucose and 22 mM fructose (mMRS). Cultures were subcultured in wort medium (20% wort extract in water v/v) for 72h to obtain a cell count of 10^6 CFU/mL. The wort medium was inoculated with LAB and fermented at 35°C for 72 h. During fermentations, bacterial growth was monitored by measuring pH and cell counts.

2.3 Oligosaccharide synthesis with *W. cibaria* 10M in orange juice

Blend fermentation was performed using *Weissella cibaria* 10M. Five blends containing mixtures based on the malt extract and orange juice concentrate were prepared as shown in Figure 1. A control containing only orange juice was also used. The pH values of each blend were measured with a pH meter. The pH was adjusted using 10% (w / v) $NaHCO_3$ obtained at a local supermarket. Blends were inoculated with 10% (v / v) of *W. cibaria* 10M in wort broth and incubated at 35 ° C for 24h. LAB cell counts and pH were determined after inoculation and fermentation. The number of colony-forming units (CFU) was determined by standard microbiological plating assays. Bacteria were also plated on mMRS-sucrose to confirm that fermentation microbiota consisted of dextran-producing *W. cibaria* 10M (Figure 2).

Glucose, fructose and sucrose were quantified in an Agilent 1200 series LC system (Agilent Techologies, Palo Alto, CA) with a Supelcosil LC-NH_2 column (250mm×4.6mm, 5um, Sigma Aldrich) coupled to a refractive index (RI) detector. Samples were diluted with acetonitrile / water (50:50, v/v) and eluted with acetonitrile/water (70:30, v/v) at a flow rate of 0.8 mL min^{-1} and 30°C. The reaction products were quantified using glucose, fructose and sucrose as external standards.

2.4 Determination of oligosaccharides

Oligosaccharides in the supernatant of fermented blends were analyzed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Samples were diluted 100 times with water, filtered, and separated on a Carbopac PA20 column (Dionex, Oakville, ON, Canada). Water (A), 0.2*M* NaOH (B) and 1*M* NaAc (C) were used as solvents at a flow rate of 0.250 mL/min, with the following gradient: 0 min, 68.3% A, 30.4% B and 1.3% C; 25 min, 54.6% A, 30.4% B and 15.0% C; 28min, 50% A and 50% C; 31min, 10% A, 73% B and 17% C, followed by re-equilibration. Maltose, isomaltose and panose were used as external standards.

Mass balance for oligosaccharide quantification and consumed sugars (total oligosaccharides, sucrose, glucose and maltose consumed, and released fructose) was carried out at times 0 and 24 of fermentation. The sugar concentrations were calculated using equations from HPLC quantification obtained from the calibration curves.

2.5 **Quantification of metabolites**

The supernatants of fermented juice were diluted 100-fold and analyzed in duplicate. To analyze organic acids, ethanol and monosaccharides, 50 µl of perchloric acid (70%) was added to 1.0 ml of supernatants and incubated at 4 °C overnight. Solids were removed by centrifugation and samples analyzed by HPLC with an Aminex HPX 87 H columns (BioRad) (Dlusskaya et al. 2008). Lactate, acetate, citric acid, fructose, mannitol and ethanol (all from Sigma) were used as external standards.

2.6 Sensory evaluation by a consumer panel

Sensory evaluation was assessed for adherence to ethical guidelines and approved by the Research Ethics Board of the University of Alberta (Protocol 00061564). The fermented blends were kept in a refrigerator at 5ºC for up to 24 h after fermentation. Five 40 mL juice blends (Figure 1) were placed separately in covered plastic cups labeled with random 3-digit numbers and randomly assigned to each member of the consumer panel. Drinking water was provided for subjects to rinse their mouth between blends.

Sixty panelists were recruited from staff and students at the University of Alberta. Demographic and personal information such as age, sex, and drinking habits were collected. The evaluation of juice sensory attributes included total acceptance, taste and acidity, scored on a Hedonic Scale. Each score rated the samples on a 9-point scale from 1 (lowest) to 9 (highest). The just about right (JAR) test was used to quantify the presence of acidity and sweetness. The JAR question format aimed to determine the best intensity of a sensory attribute by asking consumers to assess whether this attribute is too strong or too weak according to their preference (Jaeger et al., 2015).

2.7 Statistical Analysis

Results were expressed as mean values and standard deviation of independent experiments performed in triplicate. Data were analyzed using one-way analysis of variance (ANOVA). The SigmaPlot program, version 13.0, was used to test the significance level at 5% probability ($p<0.05$). Tukey's test was applied to identify significantly different means.

3. RESULTS

Sucrose promotes oligosaccharide formation via the dextransucrase activity of *W. cibaria* 10M; however, to increase sucrose concentration and favor isomalto-oligosaccharide production, different concentrations of sucrose and malt extract containing maltose were added. The pH of the blends was adjusted with sodium bicarbonate 1% (w/v) to values between 5.0 and 7.0.

During blend fermentation, *W. cibaria* 10M increased from 10^6 CFU/mL to 5.4 x 10^8 CFU /mL after 24 h of growth (Table 1). The pH of blends 3 and 4 declined from 5.5 to 3.9 after 24 h fermentation with *W. cibaria*; this acidification profile is described in the sensory analysis.

3.1 Production of isomalto-oligosaccharides

The oligosaccharide patterns of fermented blends are shown in Figure 3. The non-fermented *W. cibaria* blend used as control contained oligosaccharides. Oligosaccharide, panose and isomaltotriose formation was observed, especially for blends 1 and 2. In all blends, formulations show the presence of oligosaccharides with higher degrees of polymerization at the peaks (DP4, DP5, DP6) (Figure 3).

Sucrose was consumed during fermentation when compared to the no-fermentative process (control). The formulation containing 40% sucrose (Figure 3B), with a consumption of 74% sucrose and almost complete maltose uptake (95%) (Figure 3C), produced 10 g/L of panose and 4 g/L of isomaltotriose, and a series of peaks were produced by dextransucrase from *Weissella cibaria* 10M.

In blends composed mainly of malt extract and sucrose, oligosaccharide formation by dextransucrase activity was more evident than when orange juice was used (Figure 3D). The beverage containing only malt and sucrose was sufficient to produce more oligosaccharides, and there was no significant difference in acceptance

250 when compared to the blend containing orange juice. This reinforces alternative
251 routes of isomalto-oligosaccharide generation in order to improve production and
252 acceptability.

253 The interference of pH is critical in this process, although the adjustment
254 made with sodium bicarbonate may not be sufficient to provide suitable
255 oligosaccharide synthesis. Small concentrations of bicarbonate caused flavor changes
256 in the blends, significantly increasing bitterness, which would certainly increase
257 rejection.

258 Residual sugars were quantified by HPLC to determine the amount of
259 oligosaccharides formed by mass balance. Table 2 presents the isomalto-
260 oligosaccharide concentrations produced, as well as the consumption and release of
261 reducing and non-reducing sugars at time 0 and during fermentation.

262 The formulations containing malt extract and sucrose at different proportions
263 exhibited higher isomalto-oligosaccharide content, and these blend showed good
264 acceptability and suitable sweetness and acidity. Sucrose and maltose were consumed
265 during fermentation, demonstrating the transglycosylation or acceptor reaction.
266 Fructose formation by *W. cibaria* in blends can be attributed to its release from
267 sucrose in the dextransucrase reaction (Table 2).

268 An approximate maltose conversion rate of 94% and 92% occurred in the
269 fermentations of blend 2 and 3, respectively. The fermentations were effective for the
270 transfer reaction because more than 80% of the acceptor sugars (sucrose and maltose)
271 were consumed after 24 h of fermentation in blend which produced oligosaccharides.

272 In the presence of sucrose and a suitable acceptor, acceptor carbohydrates
273 compete with dextran to produce oligosaccharides and low molecular weight dextran
274 (Robyt and Eklund, 1983; Heincke et al., 1999; Hu et al., 2017). The high maltose

concentration in blends containing malt extract thus supported oligosaccharide formation at the expense of dextran synthesis. The primary product of the acceptor reaction with maltose panose, which is further elongated to form panose-series oligosaccharides.

The oligosaccharides obtained by the acceptor reaction have several applications potentials in the food science. Panose-series oligosaccharides, for example, can be used as a non-cariogenic sweetener in foods and beverages, as well as a food supplement since long chain IMO are indigestible and have prebiotic properties (Goffin et al., 2011; Hu et al., 2017).

Dlusskaya et al., (2008) and Zaninni et al. (2013) confirmed that maltose depletion indicates its role as acceptor sugar for dextransucrase in sucrose-supplemented wort medium. In the presence of maltose, *W. cibaria* produces panose and higher oligosaccharides of the panose series, with concomitant accumulation of fructose during fermentation, indicating almost complete conversion of sucrose (Zannini et al., 2013; Hu et al., 2017). Additionally, as sucrose levels increase, one of its hydrolysis products (glucose) acts as a building block for exopolysaccharide production, while maltose acts as an acceptor sugar for glucansucrases catalyzing the transferase reaction (Galle et al. 2010).

Isomalto-oligosaccharides are one of the most common oligosaccharides used in several countries as functional food because of their excellent nutritional properties and prebiotic activity (Zhang et al., 2013; Yan et al., 2018). The acceptor reactions of dextransucrase have also been used to synthesize isomalto-oligosaccharides (Hu et al., 2017). The enzymatic cleavage of polymers is an alternative to selectively obtain high-yield oligosaccharides (Bertrand et al., 2014); however, this approach is not compatible with fermented foods.

Previous reports show production of isomaltooligosaccharides via the dextransucrase activity of *Leuconostoc* spp. in fermented malt beverages (Dlusskaya et al., 2008; Zanninni et al., 2013); however, the beverages were not evaluated by sensory analysis.

3.2 Acid content during fermentation

Table 3 shows the organic acid content in the fermented blends. *W. cibaria* 10M produced lactic acid, acetate and ethanol in all blends, but citrate was not consumed.

The acid content in the fermented product must be analyzed to ensure beverage quality. LAB produce several functional compounds, including organic acids and antimicrobial substances. This antimicrobial activity is caused primarily by the production of lactic, acetic, formic, caproic, propionic, butyric and valeric acids. Organic acids are impacted by acidification of the environment and the antimicrobial effect of their non-dissociated molecule form, which is pH dependent. With respect to the metabolite products of lactobacilli, lactic and acetic acids are regarded as the main organic acids, which display antimicrobial behavior (Zalán et al., 2010).

CO_2 is another compound generated in the heterofermentative metabolism of hexoses (Björkroth et al., 2002). Lactose is the main metabolism product when fermentable carbohydrates are abundant (Gänzle, 2015). Heterofermentative LAB use the phosphoketolase pathway for carbohydrate metabolism. The energy yield of the pathway is only one ATP per glucose, and most heterofermentative LAB grow poorly with glucose as sole carbon source (Gänzle, Vermeulen and Vogel, 2007).

The blends contained other substrates such as maltose, sucrose and fructose, as well as alternative electron acceptors. Cofactor recycling by heterofermentative LAB impacts food quality because acetate production increases and both redox potential

and the antioxidant capacity of fermented beverages are strongly influenced. Acetate exhibits antibacterial and antifungal activity, affecting flavor and contributing to the sour taste. Excessive acetate formation can spoil a number of beverages (Garai-Ibabe et al., 2008).

Sucrose addition is important in producing sufficient amounts of exopolysaccharides; however, this may result in excessive acetate production, and the use of *Weissella* spp. that are unable to utilize maltose or fructose as electron acceptor is preferred; in addition, most *Weissella* spp. do not reduce fructose in mannitol (Galle et al., 2010).

3.3 Sensory evaluation

Sensory analysis was conducted to determine beverage quality and acceptance. Demographic data showed that the consumer panel was composed mainly of women (55%), with mean age between 18-29 years, and 75% of panelists reported regularly consuming fresh fruit juice (2-3 times a week). Fresh orange juice was consumed every week by 48% of the individuals, while 23% reported drinking carton or sugar-added juice.

In terms of general acceptability, the control sample obtained a significantly higher ($p>0.05$) score than fermented juices. The sensory score was highest (6.4) in fresh orange juice on a 9–point hedonic scale. Fermented malt extract (80%) and sucrose (20%) received a sensory score of 5.1 (Table 4). In this condition, fermentation decreases sweetness, corroborating the just about right test (Figure 7A), with sweetness close to ideal, which may have improved the taste.

The lowest score for flavor, acidity and global acceptance was awarded to blend 4, which contained orange juice, in addition to malt extract and sucrose. Thus,

consumers found the taste of the juice and malt extract mixture undesirable. Baking soda may have interfered more in this combination, resulting in a more bitter taste, as evidenced by the just about right test (Figure 4B).

In the food industry, a safe alkalizer such as baking soda is often added to control pH, helping to maintain product stability and ensuring specific microbial growth conditions. The low $NaHCO_3$ concentration used may be feasible for large scale production.

When asked about their drink preference, 62% of panelists chose fresh orange juice. Orange juice likely received higher scores because it is well known and widely consumed. However, 28% of consumers preferred the beverage containing only an 80:20 proportion of malt extract and sucrose.

Developing innovative products that satisfy various groups of consumers is essential to adding value to the food market. A popular method for hedonic assessment and product diagnosis is the just-about-right (JAR) test (Zhi, Rao and Shi, 2016). The JAR scale ratings for sweetness and acidity are illustrated in Figure 4.

High sweetness ratings for blends 1, 3 and 4 indicate sucrose addition and fermentation by *Weissella*. Dextransucrase releases glucose and fructose from sucrose, thereby enhancing sweetness. Blend 2, containing malt extract and 20% sucrose, and blend 5, containing orange juice, were highly rated for “just about right” sweetness. Samples 1, 3 and 4 also exhibited low acid content; blends 2 and 5 obtained the highest rating for “just about right” acidity. Together with sweetness scores, this indicates that the balance between a sweet and sour taste was appropriate in these beverages. Blend 2 also contained a high proportion of isomalto-oligosaccharides. The fermentative metabolism of *Weissella* also displayed these properties, producing lower acid concentrations than other microorganisms. Galle et

al. (2010) reported that low acid formation levels are beneficial in food processing due to the unpleasant flavor associated with higher amounts.

4. CONCLUSIONS

The present study showed that beverages with fermented orange juice and malt extract and fermented malt extract with sucrose are viable substrates for enzymatic synthesis of isomaltooligosaccharides, resulting in high concentrations at different degrees of polymerization. They represent a cost-reducing alternative in the manufacture of probiotic drinks.

The result of the fermented juices treated with varying levels of malt extract and sucrose demonstrated isomaltooligosaccharide production, especially when we used malt extract (16%) and sucrose (4%) fermented with *Weissella cibaria*.

The transglycosylation activity with 0.6 g / L of maltose at pH 5.5 and optimum temperature of 35 ° C was important, since oligosaccharides generated primarily panose and isomaltotriose.

Taste, acidity and general acceptability were better for the control sample (natural orange juice), followed by the fermented sample with malt extract and sucrose, which was also well accepted. The sensory results of the just about right test showed that samples had suitable sensory properties in terms of acidity and sweetness, good parameters to assess juice quality.

Enzymatic synthesis of isomaltooligosaccharides produced a drink with reduced sugar content, resulting in low-calorie juice, combined with good sensory characteristics, such as acidity and sweetness, contributing to increasing the functional food market.

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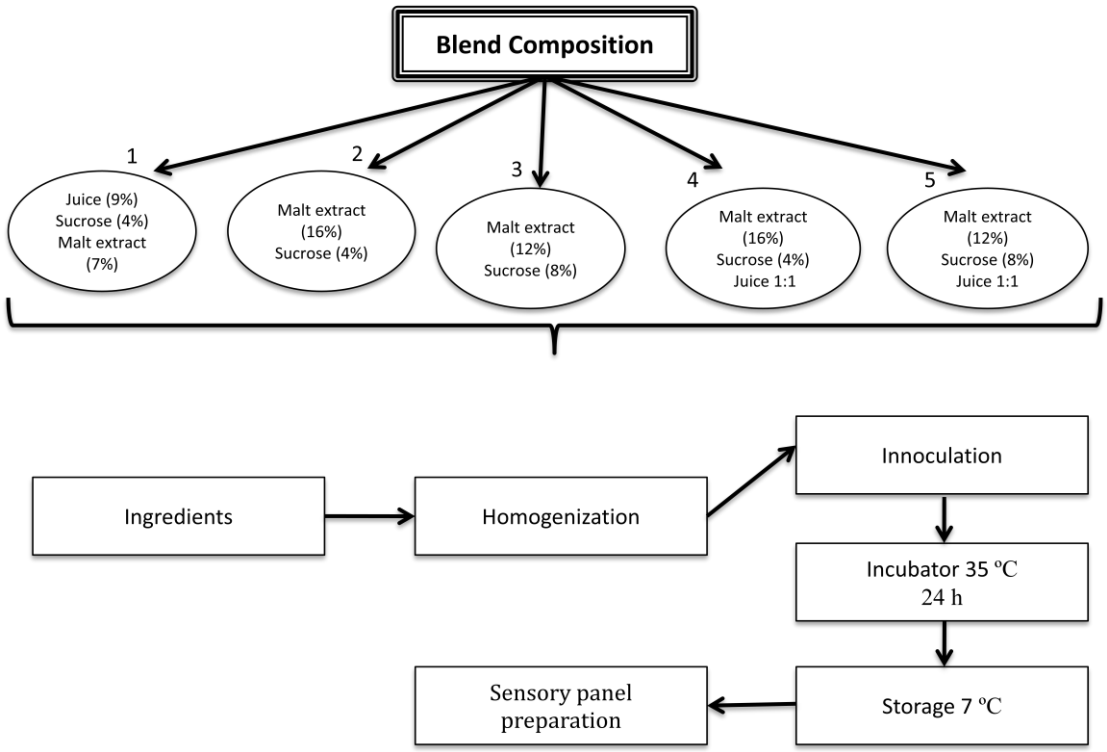

Figure 1. Blend composition and manufacturing process.

543 Table 1. Cell count of *Weissella cibaria* for different blend during the fermentation.

Time of fermentation (h)	Log $CFUg^{-1}$				
	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
0	6.0	6.5	7.4	6.1	6.1
12	6.3	7.1	8.0	7.5	7.2
24	8.2	7.9	8.7	8.5	8.1

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547 Table 2. Effect of acceptor carbohydrates on oligosaccharide yield in the acceptor
548 reaction.

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Blends	Sugar g/L	Time 0	24 h of fermentation	Sugar consumption (%)
1) Juice (9%) with 8% sucrose and 7% malt	Gluc	3.0 ±0.01	6.0 ±0.01	-
	Fruc	43±0.01	14.0 ±0.02	-
	Suc	67.2 ±0.21	9.7 ±0.20	85
	Malt	12 ±0.04	16.0 ±0.03	33
	Pan	-	12.0 ±0.03	-
	Isomalt	-	6.0 ±0.02	-
2) Malt extract (16%) with sucrose 4%	Gluc	14 ±0.34	5.2 ±0.08	-
	Fruc	6.8 ±0.05	16.0 ±0.02	-
	Suc	19.4 ±0.08	8.0 ±0.01	59
	Malt	36 ±0.02	2.0 ±0.03	94
	Pan	-	19.4 ±0.15	-
	Isomalt	-	6.0 ±0.02	-
3) Malt extract (16%) with sucrose 8%	Gluc	19 ±0.01	24.6 ±0.03	-
	Fruc	46.0 ±0.03	15.2 ±0.05	-
	Suc	10.2±0.04	2.0 ±0.03	80
	Malt	31 ±0.03	2.6 ±0.01	92
	Pan	-	10.0 ±0.11	-
	Isomalt	-	4.0 ±0.07	-
4) Malt extract (16%), suc. (4%) Juice 1:1	Gluc	38 ±0.24	36.8 ±0.46	-
	Fruc	54 ±0.04	19.0 ±0.01	-
	Suc	16.7 ±0.08	3.3 ±0.02	80
	Malt	16 ±0.08	5.2 ±0.01	67.5
	Pan	-	8.0 ±0.03	-
	Isomalt	-	4.0 ±0.04	-
5) Malt extract (12%), suc. 8% Juice 1:1	Gluc	27±0.08	53.8 ±0.58	-
	Fruc	49±0.01	18.6 ±0.03	-
	Suc	45 ±0.01	5.6 ±0.03	88
	Malt	17±0.01	6.0 ±0.08	65
	Pan	-	4.0 ±0.03	-
	Isomalt	-	4.0 ±0.02	-

550 *Gluc: glucose; Fruc: fructose; Suc: sucrose; Malt: maltose; Pan: panose; Isomalt:*
551 *isomaltotriose*
552

553 Table 3. Production of organic acid (mmol/L) in the fermented blends using orange

554 juice.

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Blends	Lactic acid	Acetate	Ethanol	Citric acid
1) Fermented Juice (9%) with 4% sucrose and 7% malt	7.6 ±0.14	9.1 ±0.06	0.5±0.01	96.7±0.10
2) Fermented malt extract (16%) with sucrose 4%	87.3 ±3.45	38.2±12.8	14.2±0.00	0
3) Fermented malt extract (12%) with sucrose 8%	36.8±0.52	130.5±1.93	3.1±0.02	0
4) Fermented malt extract (16%), suc. (4%) Juice 1:1	70.2±0.79	82.1±2.61	44.5±0.06	15.9±±0.12
5) Fermented malt extract (12%), suc. 8% Juice 1:1	55.8±2.10	52.3±3.37	50.3±0.01	74.7±0.08

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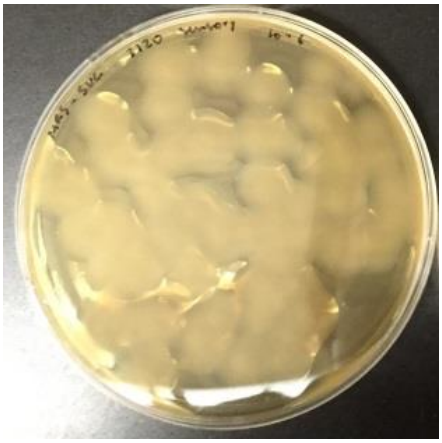
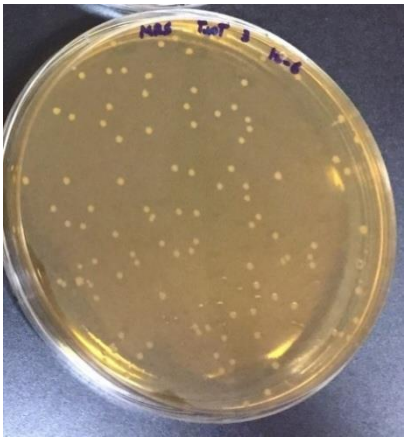
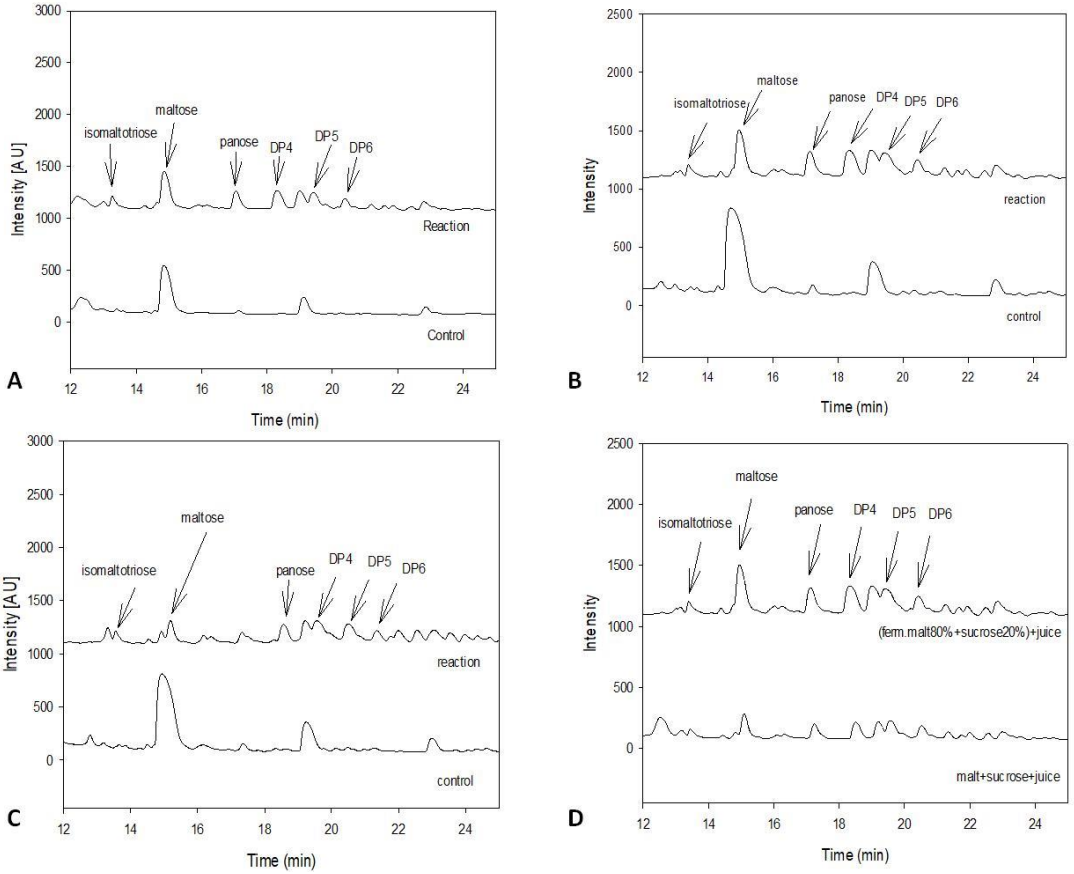
A.  B. 

Figure 2. (A) Exopolysaccharide (EPS) production by *Weissella cibaria* 3120 on 10% sucrose-supplemented MRS agar in the form of slimy colony morphology, and (B) no EPS production by the same strain in the absence of sucrose.



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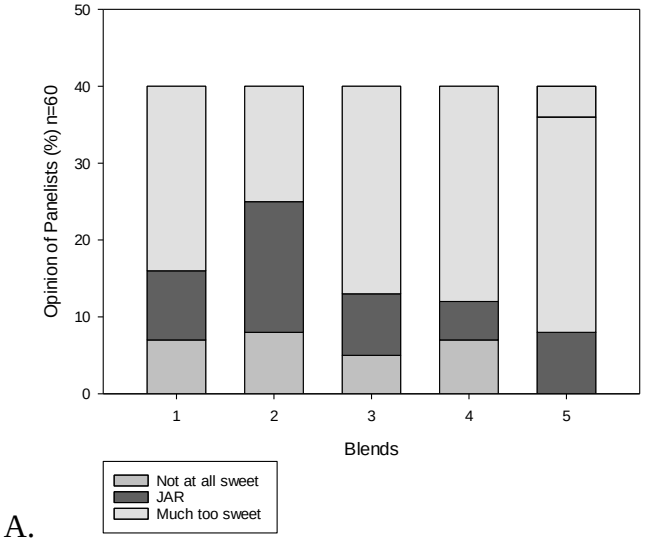
564 Figure 3. Separation of oligosaccharides in juice blends with HPAEC-PAD. (A) 9%
565 orange juice (w/v), 7% malt extract (w/v) and 4% sucrose (w/v). (B) 12% malt
566 extract and 4% sucrose (w/v). C. 12% malt extracts and 8% sucrose (w/v). D. 16%
567 malt and 4% sucrose + orange juice 1:1.

568 Table 4. Sensory scores for taste, acidity and overall acceptance on a 9-point hedonic

569 scale of fermented blends treated with sucrose, malt extract and orange juice.

Sensory attributes	1) Juice (9%) 4% sucrose 7% malt	2) Malt extract (16%) sucrose 4%	3) Malt extract (12%) sucrose 8%	4) Malt extract (16%), suc. (4%) Juice 1:1	5) Malt extract (12%), suc. 8% Juice 1:1	Fresh Orange juice
Taste	3.58 ± 1.87^{c}	3.90 ± 1.92^{c}	4.90 ± 1.71^{b}	3.95 ± 1.43^{bc}	3.07 ± 1.71^{c}	6.42 ± 1.48^{a}
Overall	3.48 ± 1.88^{cd}	3.88 ± 1.90^{c}	4.83 ± 1.71^{b}	3.85 ± 1.42^{cd}	3.07 ± 1.64^{d}	6.30 ± 1.41^{a}
Acidity	4.00 ± 1.77^{d}	4.30 ± 1.73^{cd}	4.92 ± 1.44^{b}	5.05 ± 1.28^{bc}	3.77 ± 1.84^{d}	6.15 ± 1.38^{a}



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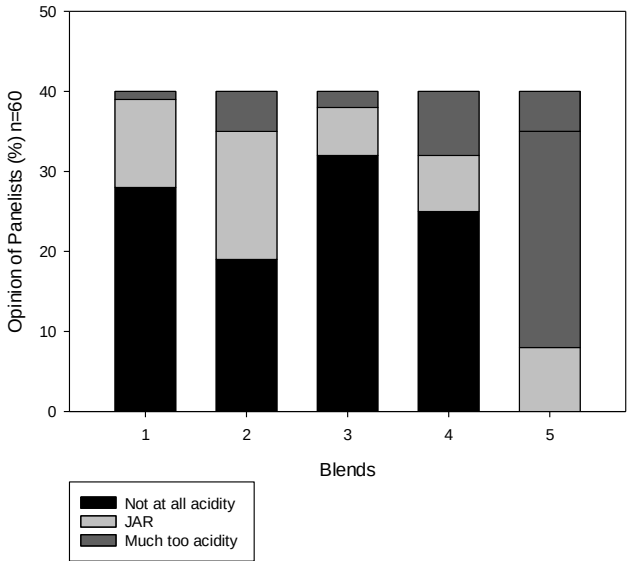

B.

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572 Figure 4. Panelists' opinion regarding Just about right rating for (A) Sweetness and

573 (B) Acidity of fermented orange blends.

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