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Fructooligosaccharides Stability during the Processing and the Shelf Life of an Aseptically Packed Commercial Pineapple Nectar

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Abstract The purpose of this research is to evaluate the stability of the added fructooligosaccharides during the processing and the shelf life of an aseptically packed commercial pineapple nectar. With this objective, a pineapple nectar was produced in a fruit juices factory, being enriched in fructooligosaccharides (0.6%). The pineapple nectar was sterilized by heat and aseptically packed. The storage of the pineapple nectar was at room temperature. A sample of six bottles of nectar was taken before nectar processing and once packed, at time intervals during the one-year storage, also was taken a sample of six packs of nectar. The following analyses were carried-out: soluble solids, pH, turbidity, colour, content in fructooligosaccharides, sucrose, glucose and fructose, and microbiology. The results indicate that soluble solids, pH, turbidity and colour parameters keep constant during the processing and the storage. The microbiology of the packed nectar indicates the effectiveness of the thermal treatment. Fructooligosaccharides and sucrose are stable during the high temperature-short time thermal treatment and during the aseptic packaging, while they are instable during the storage. After one year, the concentration of fructooligosaccharides and sucrose was the 14% and 30% of the initial, respectively. Considering the instability of the fructooligosaccharides in acid medium, fruit beverages producers must adjust the initial content in order to keep some levels of fructooligosaccharides during the shelf life, even at its end. This result also suggests that fructooligosaccharides could be more adequate to enrich solid food than liquid food, if the shelf life is long.

Keywords: *fructooligosaccharides, sugars, shelf life, pineapple, nectar, aseptic packaging*

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1. Introduction

The consumers are more and more selecting healthier foods, such as functional foods. This has targeted the food industry to the development of foods and beverages containing prebiotics and other functional ingredients [1,2,3]. The use of fruit juices and fruit derived beverages as a via for prebiotic carbohydrate intake is a promising area. Research on the carbohydrate stability is essential to assure the functional properties of the final product, even at the end of its shelf life [1,3].

It has been reported the prebiotic character of the fructooligosaccharides, since they are degraded by probiotic microorganisms at the human gut [1,4,5], with the subsequent human health benefits such as increase of the immunity, reduce the risk and severity of gastrointestinal infection, reduce metabolic disorders related to obesity and promote the anti-cancer effect [2,6]. The presence of probiotic microorganisms in the human gut is very desirable, since probiotics help to keep a healthy status of the gut [7] and stimulate the growth of bifidobacteria in the human colon [4,8]. The fructooligosaccharides encourages the growth of friendly bacteria, which in turn discourages the growth of potentially putrefactive microorganisms in the colon, resulting in a healthy gut environment [4,8].

The fructooligosaccharides are naturally present in some food and also can be added to food and beverages during the production of those functional products at industrial level [9,10,11]. Fructooligosaccharides suffer hydrolysis in the acidic liquid medium of some fruit beverages [7,12] with loss of its prebiotic character.

There are two main industrial factors against the integrity of the fructooligosaccharides: the thermal treatment at acid pH during processing before packaging and the storage time until consumption [1,13,14]. The non-thermal technologies could prevent the hydrolysis of the fructooligosaccharides, because of the milder operating conditions that are applied [15]. While it has been carried out studies on the effect of the thermal and the non-thermal technologies on the integrity of the fructooligosaccharides [14-21], the studies on the integrity of the fructooligosaccharides during the storage are scarce, and the storage time tested was no longer than six months [14,22,23,24,25].

The aim of this work is to evaluate the stability of the fructooligosaccharides during the processing and the one-year shelf life of an aseptically packed commercial pineapple nectar, in order to get knowledge of what is an initial adequate concentration of fructooligosaccharides, that still allows its presence in the packed nectar one year later of storage.

2. Material and Methods

2.1. Fructooligosaccharides

Fructooligosaccharides (Orafti® P95 oligofructose) were from Beneo (Mannheim, Germany).

2.2. Industrial Elaboration of the Aseptically Packaged Commercial Pineapple Nectar and Sampling

The pineapple nectar was elaborated, processed and aseptically packaged in a fruit juice producer factory located in Murcia, Spain, which is not a pineapple producing country. Pineapple juice to make industrial nectars is usually imported as concentrate juice. Pineapple concentrate juice (65°Brix) from Del Monte (Costa Rica) was diluted ten times (6.5°Brix) with calcium-free potable water and fructooligosaccharides (0.6 %) were added, resulting 7.0°Brix. The pH of the nectar was adjusted to 3.5 with citric acid (E-330). This is the pineapple nectar before processing analysed in this research by sampling of six 1 liter bottles. The pineapple nectar was processed by thermal heating (flash pasteurization) in a tubular heat exchanger at 115°C for 15 sec and cooled to 30ºC. Then the sterile pineapple nectar was aseptically packed in Tetrapak (Lund, Sweden) packs of 333 ml. These packs were stored at room temperature and six packs were sampled at 3 days of storage, 10 days, 18 days, 30 days (1 month), 43 days (1.4 months), 58 days (1.9 months), 91 days (3 months), 122 days (4 months), 228 days (7.5 months), 283 days (9.3 months) and 374 days (12.3 months). The samples were analysed for physico-chemical characteristics, content of sugars and microbiology.

2.3. Physico-chemical Characteristics

2.3.1. Soluble Solids, Humidity and Water Activity

The soluble solids (ºBrix) were measured with an Atago N-1E (Tokyo, Japan) refractometer directly in the samples of the pineapple nectar. The humidity (%) of the nectar was estimated as 100 minus the soluble solids and the water activity was measured with a Novasina apparatus (Novasina, Lachen, Switzerland).

2.3.2. pH

The pH was measured with a Crison pHmeter (Barcelona, Spain) directly in the samples of the pineapple nectar.

2.3.3. Turbidity

The turbidity was measured directly in the samples of the pineapple nectar with a Pye Unicam spectrophotometer (London, United Kingdom). The turbidity is the percentage of transmittance at 660 nm of the pineapple nectar sample against a blank of pure water. T_{660} of the pure water is 100%. As the sample is much cloudy, the T_{660} is much close to 0%.

2.3.4. Colour

The colour was measured using a hand held tristimulus reflectance colorimeter (Chromameter II CR-200, Minolta, Osaka, Japan). The results were expressed as International Commission on Illumination (CIE) CIELab units: L* (lightness), a* (redness) and b* (yellowness). Triplicate readings were taken at room temperature in the samples of the pineapple nectar.

2.4. Chromatographic Analysis of the Fructooligosaccharides, Sucrose, Glucose and Fructose

The fructooligosaccharides and sugars determination [14] was carried out by High-Performance Liquid Chromatography (HPLC), using a CHO-682 column (30 cm x 7.8 mm; Transgenomic, San Jose, CA, USA) and refractive index detection (LaChrom, Merck, Darmstad, Germany). The fructooligosaccharides (Orafti® P95 oligofructose) from Beneo (Mannheim, Germany) and pure sugars from Merck (Darmstad, Germany) were used as external standards for calibration. More details on sample preparation and HPLC analysis were published previously [26].

2.5. Microbiological Analysis

To establish a profile of the microbiological population of the pineapple nectar, molds and yeasts were quantified. The culture medium used was Dichloran Glycerol Agar (DG18) (Oxoid, Madrid, Spain), supplemented with glycerol (Sigma, St. Louis, MO, USA). The medium was sterilized in autoclave at 121ºC for 20 minutes. The samples were analyzed in duplicate. From each sample, a portion of 10 ml was taken with sterile spoons and then mixed with 90 ml of buffered pectone water (BPW) (Oxoid, Madrid, Spain), obtaining dilution 10^{-1}. From this dilution, successive dilutions were obtained putting into tubes containing 9 ml of BPW and 1 ml of previous dilution. The seeding process of the DG18 plates was carried out in a laminar flow hood. For the analysis of mold and yeast, plates were incubated at 25°C for 5-7 days. After this, the manual recount of the plates was carried out, expressing the result as log cfu/g.

2.6. Statistical data processing

All analyses were made in triplicate. The batch of the sampled pineapple nectar was of n=6 bottles or packs and N=72 (one year). The values shown in the Table 1 correspond to the average value of the analysis of each parameter in the different samples. The average values and coefficients of variation (cv, %) were calculated with the Excel (Microsoft Office 2019) software. The industrial processing and the storage time were considered as treatments. The effect of the treatments on the physico-chemical characteristics of the nectar and its content in sugars was determined by one-way ANOVA. LSD's homogeneity means test (P<0.05) was used. The statistical computer program used was Statistix 8 for Windows (Analytical Software, Tallahassee, FL, USA).

3. Results and Discussion

3.1. Physico-chemical Characteristics of the Pineapple Nectar

The Table 1 provides the values of soluble solids, pH, turbidity and colour parameters in the aseptically packed commercial pineapple nectar, before processing and during one year of sampling, at different time intervals, of the nectar packs stored at room temperature.

3.1.1. Soluble Solids

The thermal treatment and packaging did not change with statistical significance the soluble solids of the pineapple nectar (6.8 and 7.0ºBrix, respectively, Table 1). Concerning the one-year storage, there was a statistically significant difference after 7.5 months (6.4ºBrix), and also at the end of the storage (6.3ºBrix, 12.3 months). Reference [25] has reported a soluble solids content of 18.5ºBrix in a pineapple juice enriched with 2% inulin and 2% sucrose, which change from 18.5ºBrix (day 1) to 18.2ºBrix (day 28) during four weeks of storage at 4ºC. The fruit content of the pineapple nectar is 50%. This explains the lower content of soluble solids in the nectar than in the pineapple juice.

3.1.2. pH

The pH was also a bit changing (Table 1). For unknown reasons, except for error in the measurement, the thermal processing and packaging caused a small decrease in the pH, from 3.5 to 3.2, with statistical significance. The pH fluctuates during the storage between 3.2 and 3.6, which is really a very small difference, although with statistical significance. Reference [25] has reported a pH of 3.7 in a pineapple juice enriched (2%) with inulin, which keep constant during four weeks of storage at 4ºC.

3.1.3. Turbidity

The pineapple nectar is a very cloudy beverage (Table 1), since all turbidity values are between 0.2 and 0.4% of transmittance at 660 nm. The change in turbidity due to processing and packaging is very small, from 0.2 to 0.4%, respectively, with significant statistical difference. This value of 0.4% keeps constant during the storage till 7.5 months, with a change to 0.3% (9.3 months) and 0.2% (12.3 months), with statistical significance. Reference [25] has reported a turbidity of 0.3 in a pineapple juice enriched with 2% inulin, which keeps constant during four weeks of storage at 4°C.

3.1.4. Colour

Concerning the colour parameter L* (luminosity), although the statistical processing of the results indicated significant differences, the values are all around 32 (Table 1). The thermal processing and aseptic packaging caused a small change from 32.0 to 32.2, respectively, with significant statistical difference. At 9.3 months of storage the L* was 31.6, and 32.6 at 12.3 months. Reference [25] has reported a value of L* of 57.5 in a pineapple juice enriched (2%) with inulin, which keeps constant during four weeks of storage at 4ºC (57.4). The colour parameter a* (redness) was constant (Table 1), always 0.0 or 0.1, without statistical differences, and concerning the colour parameter b* (yellowness), the thermal processing and aseptic packaging caused a small change from -4.8 to -5.0, respectively, with significant difference, being -5.3 at the end of the 12.3 months of storage. Reference [25] has reported a value of a* of 1.3 and a value of b* of 20.4 in a pineapple juice enriched (2%) with inulin, which keeps constant during four weeks of storage at 4ºC. Differences between nectar and juice are due to a different pineapple content, and also to different thermal treatment, flash pasteurization for the pineapple nectar and long pasteurization (80ºC for 20 min) for the pineapple juice.

Table 1. Values of the soluble solids, pH, turbidity and colour parameters in the aseptically packed commercial pineapple nectar, before processing and during one year of sampling at different time intervals of the nectar packs stored at room temperature (n=6, N=72 (one year))

Time of sampling	S. solids (ºBrix)	pH	Turbidity (T_{660}%)	Colour: L*	Colour: a*	Colour: b*
Before processing	6.8 a	3.5 a	0.2 c	32.0 c	0.0	-4.8 a
3 days	7.0 a	3.2 c	0.4 a	32.2 b	0.0	-5.0 b
10 days	6.9 a	3.4 b	0.4 a	32.0 c	0.1	-4.9 a
18 days	7.0 a	3.5 a	0.4 a	32.5 a	0.1	-4.8 a
1 month	6.8 a	3.4 b	0.4 a	32.3 a	0.1	-4.9 a
1.4 months	6.9 a	3.5 a	0.4 a	32.5 a	0.0	-5.2 c
1.9 months	6.9 a	3.5 a	0.4 a	32.5 a	0.0	-5.2 c
3 months	6.9 a	3.4 b	0.4 a	32.5 a	0.0	-5.3 d
4 months	6.8 a	3.4 b	0.4 a	32.3 a	0.0	-5.3 d
7.5 months	6.4 bc	3.5 a	0.4 a	31.5 d	0.0	-5.0 b
9.3 months	6.5 b	3.4 b	0.3 b	31.6 d	0.1	-5.1 b
12.3 months	6.3 c	3.6 a	0.2 c	32.6 a	0.1	-5.3 d

[a,b,c,d]: different letters within the same column differ significantly (P<0.05).

3.2. Microbiology

Pineapple nectar presented a high humidity (93%) and a high water activity (0.95) as beverage, and could be vulnerable to some microbial alterations. However, pineapple nectar will be safe from development of the majority of bacteria, since its pH is lower than 4.0. Due to the low pH values of pineapple (3.5), the main typical flora consists of molds and yeasts. Microbiological analyses revealed that aseptically packed pineapple nectar samples presented a total number of molds and yeasts of 0 log cfu/g, indicating that the sterilization of the product and the aseptic packaging were correct. This result of 0 log cfu/g was the same for all the samples from the one-year storage of the pineapple nectar packs, and it is in agreement with the microbiological stability of a pasteurized prebiotic beverage composed by cashew-apple and yacon [27] during 7.5 months of cold storage (4°C).

3.3. Content of Fructooligosaccharides, Sucrose, Glucose and Fructose in the Aseptically Packed Commercial Pineapple Nectar, during the One-Year Storage

The content of fructooligosaccharides, sucrose, glucose and fructose in the aseptically packed commercial pineapple nectar was established by high resolution liquid chromatography (HPLC). The sugar content (mg/100 ml) of the pineapple nectar before processing (thermal treatment and aseptic packaging) was as follows: FOS: 552 ± 23, Sucrose: 1662 ± 74, Glucose: 1436 ± 68, Fructose: 1500 ± 67, Total: 5150 ± 232. The analysed content of FOS agrees with the intended (0.6%) concentration.

Using the corresponding calibration standards for each sugar, the peak chromatographic areas were converted to the corresponding concentrations in the aseptically packed pineapple nectar. The Figure 1 has been constructed obtaining the average concentration value for each sugar at each sampling storage time, and provides the change in the content of the fructooligosaccharides and other sugars in the aseptically packed commercial pineapple nectar, during its one-year storage.

The sugar content (mg/100 ml) of the pineapple nectar after processing (thermal treatment and aseptic packaging) at three days of storage was as follows: FOS: 679 ± 17, Sucrose: 1730 ± 25, Glucose: 1458 ± 22, Fructose: 1417 ± 32, Total: 5284 ± 96. Comparing the content of fructooligosaccharides before processing (data shown above) and after processing (sample of nectar after three days of storage, Figure 1), it can be seen that fructooligosaccharides are around 100% stable under the sterilization heat treatment conditions (high temperature - short time) and the aseptic packaging used. The other sugars in the nectar are also stable. This result of the stability of fructooligosaccharides is better to that (retention of 30-50%) reported by reference [28] in a berry juice enriched with fructooligosaccharides, who apply longer pasteurization conditions (90°C for 30 sec) than us (115°C for 15 sec, flash pasteurization), and also it is better to the stability (retention of 70-72%) of natural fructooligosaccharides during blanching (100ºC for 6 min) of yacon roots slices [29]. Reference [30] has also reported a high stability of oligosaccharides derived from lactulose during the processing of milk (100ºC for 30 min) and apple juice (90°C for 15 min), and reference [31] has reported a high stability of an oligofructose during the pasteurization (80°C for 20 min) of an apple juice in both glass and plastic packs. It must be stated a certain protective effect of the matrix nectar on fructooligosaccharides integrity, since the stability of fructooligosaccharides in orange juice (pH 3.5) and in a sodium citrate buffer solution with the same pH is higher in the juice than in the buffer [13]. In this sense, reference [12] has reported the instability kinetic of the fructooligosaccharides at pH 4 (buffered solution) at 110°C and up to 60 min of analysis.

The Figure 1 shows how during the storage of the aseptically packed pineapple nectar, the content of the fructooligosaccharides in the nectar tends to decrease, being at 12.3 months of storage the 14% of the initial (percentage of retention). The same trend has been observed in the sucrose content, which also decreases to a value of the 30% of the initial. The retention of the fructooligosaccharides in a pasteurized orange juice after 28 days of cold storage (4°C) was the 85% [32]. The retention of an oligofructose in a pasteurized apple juice also after 28 days of cold storage (4ºC) was the 90% [31]. These results are lower than our retention (95%) in the pineapple nectar packs, for the same period of storage (28 days) at ambient temperature. The retention of fructooligosaccharides in pineapple juice stored at 25°C during six months is 13% [22], which is similar to the 14% of retention that we found in pineapple nectar, but with the difference of the storage time, six months and one year, respectively. The instability of the fructooligosaccharides in acidic medium confirmed the results of reference [7], who stated that the fructooligosaccharides are the most acid-sensitive component. The Figure 1 also shows that the glucose and the fructose increase during the storage, since fructooligosaccharides and sucrose produce them by hydrolysis. The glucose and fructose are added to the glucose and fructose initially present in the pineapple nectar.

Reference [33] has stated that a functional beverage in order to be considered prebiotic, should contain between 1.5 and 4 g of inulin per 100 ml and a maximum daily intake of 8 g. The application of the above to the aseptically packed commercial pineapple nectar means that if it is fortified with the maximum of 4% fructooligosaccharides, after one year they will be the 0.56%. Then, it is recommendable to reduce the shelf life to around nine months, in order to have still sufficient fructooligosaccharides (> 1.5%) that allow the daily intake recommended by reference [33] and in this way maintain the functional character of the pineapple nectar enriched in fructooligosaccharides.

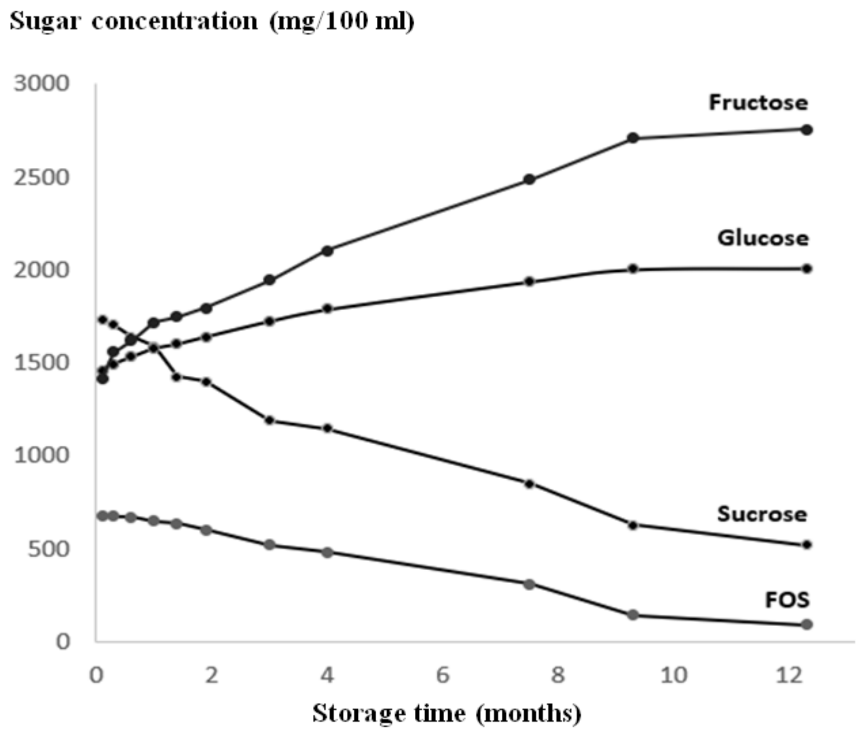

Figure 1. Content of sugars (FOS - fructooligosaccharides, sucrose, glucose and fructose) in the aseptically packed commercial pineapple nectar, during one year storage, being the sampling (N=72) at different time intervals (n=6). The storage of the packs was at room temperature. Error bars are not shown in each point of the figure, since for all points (average of six analyses) cv < 5%

4. Conclusions

The physico-chemical and microbiological analysis indicated that the aseptically packed pineapple nectar is a stable and safe beverage, since almost no changes in the analysed parameters were found during one-year storage sampling. The fructooligosaccharides are stable during the industrial elaboration, processing and aseptic packaging of the pineapple nectar, while they are instable during the long time storage of the packs. After one year, the concentration of the fructooligosaccharides was the 14% of the initial (percentage of retention). Considering the instability of the fructooligosaccharides in acid medium, fruit beverages producers must adjust the initial content in order to keep some levels of the fructooligosaccharides during the shelf life, even at its end, which should be adjusted to the real content of the prebiotic fructooligosaccharides in the commercial pineapple nectar. This result also suggests that the fructooligosaccharides could be more adequate to enrich solid food than liquid food, if the shelf life is long.

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Conflict of Interest Statement

The authors declare no conflict of interests.

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