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Application of hydrolyzed carrot pomace as a functional food ingredient to beverages

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Abstract

Utilization of a carotene-rich functional food ingredient recovered through mechanical and enzymatic breakdown of the tissue of carrot pomace was evaluated by its application to model beverages based on cloudy apple juice, aiming at sustainable carrot juice production. Contrary to synthetic β-carotene supplements, the stability of the natural α- and β-carotene in the beverages proved to be excellent after 20 and 24 weeks storage under moderate and even intense illumination at 23 and 19 °C, respectively. Neither degradation nor isomerization were observed, thus confirming the extraordinary stability of carotenes in their natural matrix. Furthermore, cloud stabilities as determined by centrifugation and real time sedimentation tests were satisfactory. Although increasing proportions of pectin only slightly delayed sedimentation of coarse cloud particles, the positive effect of pectin was more pronounced with respect to improved serum turbidity of the beverages which might be ascribed to higher serum viscosity.

Key words: Beverage technology, carrot pomace, carotene stability, cloud stability, functional food ingredient.

Introduction

In Europe and in the USA carrots (*Daucus carota* L. ssp. *sativa*) considerably contribute to human β-carotene intake. Carrot juices and blends thereof are among the most popular non-alcoholic beverages. From 1995 to 2000, German carrot juice production increased by 80 %, actually amounting to 42.2 million liters. Despite considerable improvements in processing techniques including the use of technical enzymes, mash heating, and decanter technology, about one third of the raw material remains as pomace which is usually disposed as feed or fertilizer[1]. Thus, a major part of valuable compounds such as carotenes, uronic acids and neutral sugars is still retained in the pomace[2]. In recent years, dietary supplements like ATBC (vitamin supplemented) drinks have attained intense consumer attraction. Since the incidence of lung cancer in smokers was found to be inversely correlated with high dosages of synthetic β-carotene[3,4], the former German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) recommended smokers to abstain from food and dietary supplements fortified with β-carotene[5]. According to a more recent recommendation of the BgVV daily intake of isolated β-carotene should not exceed 2 mg[6]. In contrast, intake of carotenes from fruits and vegetables was recognized as safe and beneficial. Therefore, carrot pomace is considered a valuable and yet underestimated source of natural α- and β-carotene present in their genuine proportions. In the past, only few attempts have been carried out at utilizing carrot pomace in foods such as bread[7], cake, dressing and pickles[8], and for the production of functional drinks[9], respectively. However, consumer acceptance of carrot pomace as a part of the recipe still needs to be demonstrated, especially since sensory quality may be adversely affected. Recently, a novel process for the complete utilization of carrot pomace as a source of carotenes and oligogalacturonic acids has been developed including mechanical and enzymatic breakdown of the plant matrix[10,11]. The main objective of this work was to evaluate the potential use of liquefied carrot pomace as a functional food ingredient. For that purpose a carotene-rich hydrolyzate was incorporated in a model beverage based on cloudy apple juice. Particular attention was paid to carotene and cloud stability during storage.

Experimental

Materials and reagents: Carrot pomace hydrolyzate (3.8 °Bx) with a total carotene content of 64 mg kg^{-1}, expressed as the sum of α- and β-carotene, was recovered from industrially processed carrots cv. 'Karotan' (Gemüsesaft GmbH, Neuenstadt/Kocher, Germany) as described previously[11]. The α- and β-carotene ratio was 0.57. Cloudy apple juice was kindly provided by Streker Natursäfte (Aspach, Germany). All reagents and solvents were of analytical or HPLC grade (Merck, Darmstadt, Germany). All-*trans*-β-carotene (Type II) and mixed isomers (α- and β-carotene in a ratio 1:2) were purchased from Sigma (St. Louis, MO, USA). The internal standard β-apo-8'-carotenal was obtained from Fluka (Buchs, Switzerland). Citrus pectin (Type AU 202) with a degree of esterification of 72.5 % was supplied by Herbstreith & Fox KG (Neuenbürg, Germany).

Preparation of model beverages based on cloudy apple juice: Cloudy apple juice and pomace hydrolyzate were blended to yield 50 % fruit juice content and 12 mg L^{-1} total carotene in 45 kg final product on pilot plant-scale. Soluble solids, pH, and titratable acid as well as fruit juice, ascorbic acid and total carotene content were adjusted according to commercial ATBC and breakfast drinks. After de-aeration for 10 min in a colloid mill under vacuum (100 mbar), the product was homogenized at 180 bar and de-aerated again. Weight and soluble solids (°Bx) were determined. Pectin was predissolved in water at approx. 20 °C to obtain a 10 g kg^{-1} solution which was added to the beverage at concentrations of 0.5 and 1 g kg^{-1}, respectively. Sucrose solution (68 °Bx) was added to obtain 12 °Bx in the final product. After addition of ascorbic acid (0.43 g kg^{-1}) and citric acid (2.3 g kg^{-1}), the blend was adjusted to the final concentration with water, pasteurized in a tubular heat exchanger (Ruland, Neustadt/Weinstrasse, Germany) at 90 °C for 60 s, hot filled into 0.5 L glass bottles, sealed under superheated steam (Type SLW 100 Vaporette, Schmalbach-Lubeca, Hannover, Germany), and then water cooled to room temperature. Control samples without addition of pectin and ascorbic acid were also produced, respectively. The specifications of the model beverage variants are given in Table 1.

Storage conditions of model beverages: Stability of carotenes was evaluated after 20 and 24 weeks storage of bottled samples of variant 1 and 2 under moderate and intense illumination at 23 °C and at room temperature, respectively, as shown in Table 2. Illumination intensities (lx-values) were measured with a luxmeter (Type Mavolux-digital, Gossen, Nürnberg, Germany).

Sample preparation and HPLC analysis of α- and β-carotene: Saponification of the model beverage, which was necessary due to the addition of cloudy apple juice, was carried out according to a modified procedure of Kimura et al.[12]. An aliquot of 5 mL was stirred at room temperature for 30-60 min in 60 mL of petroleum ether and 30 mL of methanolic KOH (100 g kg^{-1}) under nitrogen. The suspension was filtered through sintered glass (G_2) under reduced pressure, transferred into an amber glass separation funnel, and shaken with 50 mL of sodium chloride solution (100 g kg^{-1}). After separation, the petroleum ether layer was washed twice with water (50 mL). Emulsions were removed by adding a few drops of methanol. The extract was dried with anhydrous sodium sulfate (2 g), and petroleum ether was evaporated *in vacuo* at room temperature. The resulting residue was dissolved in 2-propanol, 1 mL of a solution of β-apo-8'-carotenal was added and made up to 5 mL. The determination of carotenes was carried out in duplicate. HPLC analysis and quantification based on internal standard calibration with β-apo-8'-carotenal was performed as described by Marx et al.[13] and Stoll et al.[11], respectively. Calculated total carotene contents were standardized to soluble solids of 12 °Bx.

Characterization of beverages:

Turbidity measurement: The turbidities of the cloudy apple juice and model beverages before centrifugation (T_0) and the turbidities of their supernatants (sera) after centrifugation (T_S; 4200 *g*, 15 min, 20 °C) were determined. The cloud stability was deduced from the relative turbidity (T_{rel}), according to Reiter et al.[14]. Turbidity was determined nephelometrically with a two-beam-photometer (Type LPT 5, Dr. Lange, Düsseldorf, Germany) using 50 mm round cuvettes. Samples were diluted with de-ionized water. Turbidity was expressed as nephelometric turbidimeter units (NTU). Additionally,

$$T_{rel} = \frac{T_S}{T_0} * 100$$

cloud stability was checked during a 170 day sedimentation test. Five bottled samples of variant 2, 3 and 4 were stored in the dark at about 18 °C, respectively. The decrease in sediment height, relative to the initial value, was calculated and plotted against storage time.

Particle size distribution: Particle size distributions of the model beverages were determined through laser light scattering with a Mastersizer E (Malvern Instruments, Herrenberg, Germany) using a condensing lens with a focal length of 300 mm. Data obtained were evaluated using supplier-provided software. Volume *D*[4,3], surface *D*[3,2], length *D*[2,1], and number *D*[1,0] mean diameters were determined for all samples.

Soluble solids: °Bx-values were determined with a digital refractometer (Type RX-5000, Atago, Tokyo, Japan).

Titratable acid: The determination of titratable acid in the model beverages was performed according to IFU method No. 3.

Color measurement: L*a*b* values of the beverages before and at the end of storage under conditions mentioned in Table 2 were determined with a Chroma-Meter (Type CR-300, Minolta, Langenhagen, Germany). The sample was poured in a Petri dish (diameter: 14 cm, height: 2 cm), and L*a*b* values were recorded ten times using standard illuminant D65.

Results and Discussion

Application of the carotene-rich hydrolyzate to model beverages: Besides several other possibilities, the most obvious application for the carrot pomace hydrolyzate is its utilization as a functional ingredient in non-alcoholic beverages, since β-carotene intake from fruits and vegetables is considered beneficial. In contrast, commercial products, e.g. ATBC and breakfast drinks, predominantly contain synthetic β-carotene formulations for provitamin A supplementation, which has been associated with adverse effects at high dosages[3,4]. Moreover, crystalline β-carotene formulations are usually based on gelatin, whereas for plant foodstuffs pectin is a more adequate hydrocolloid[15]. Consequently, model beverages consisting of 50 % apple juice (12 °Bx), 12 mg L^{-1} total carotene, 0 and 0.43 g kg^{-1} ascorbic acid, 2.3 g kg^{-1} citric acid, and varying amounts of citrus pectin were prepared. Cloudy apple juice was chosen because of its high cloud stability[16]. Analytical data of a model beverage are exemplified for variant 2 as follows: pH value of 3.2, 5.4 g L^{-1} titratable acid (calculated as citric acid), 12.2 °Bx, sugar-acid-ratio of 22.6, and 4.4 mg L^{-1} and 7.5 mg L^{-1} α- and β-carotene, respectively.

Particle size distribution of model beverages: The particle size distribution of variant 2, expressed as volume percent of the total particles is exemplarily shown in Fig. 1. Approximately 47 % of particle size ranged from 83-151 µm with a maximum of 17 % between 101 and 124 µm, 17 % occupied category 56-83 µm, and 16.8 % category 151-224 µm. Furthermore, the volume *D*[4,3], surface *D*[3,2], length *D*[2,1], and number *D*[1,0] mean diameters were found to be 117.3, 73.8, 22.9, and 6.2 µm, respectively.

Cloud stability during storage: The amount of coarse particles is a crucial issue for the cloud stability of carrot juices[14]. A cloud stabilizing potential of pectin on pulp-containing fruit beverages was attributed among others to the increasein serum viscosity[17]. Therefore, in preliminary laboratory scale experiments, citrus pectin was added in concentrations from 1 to 10 g kg^{-1} in 1 g kg^{-1} increments to evaluate cloud stability as well as mouthfeel. As described by Mensah-Wilson et al.[17], pectin addition neither affected particle size distribution nor volume mean diameter *D*[4,3]. Based on these findings beverages containing 0, 0.5, and 1 g kg^{-1} pectin were prepared in pilot plant-scale.

The centrifugation test, primarily developed for cloudy apple juice, is generally useful to predict sedimentation during storage and consequently for evaluation of cloud stability[18]. The initial turbidities of the beverages, the turbidities of the supernatant after centrifuga-

Table 1. Variation of pectin and ascorbic acid content of model beverages produced by addition of carrot pomace hydrolyzate to cloudy apple juice.

Ingredients	Variants 1	2	3	4
Pectin (g kg^{-1})	0.5	0.5	1	0
Ascorbic acid (g kg^{-1})	0	0.43	0.43	0.43

Table 2. Storage conditions of model beverages for evaluation of carotene stability after 20 and 24 weeks.

	Storage period (weeks)	Variant [a]	Temperature (°C)	Illumination intensity (lx) [b] range	average
Moderate illumination [c]	20	1	23	75-88	82
		2		75-84	80
Intense illumination [d]	24	1	16-21	2950-3800	3375
		2		2990-3670	3330

[a] Cf. Table 1
[b] 24 hrs illumination with artificial light
[c] Fluorescent lamp: L58W/25 Universal white (Osram, Munich, Germany)
[d] Four fluorescent lamps: L36W/76 Nature de luxe (Osram)

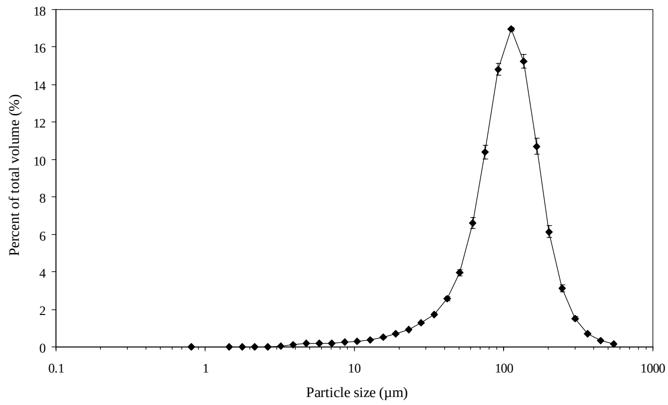

Figure 1: Particle size distribution of model beverage variant 2

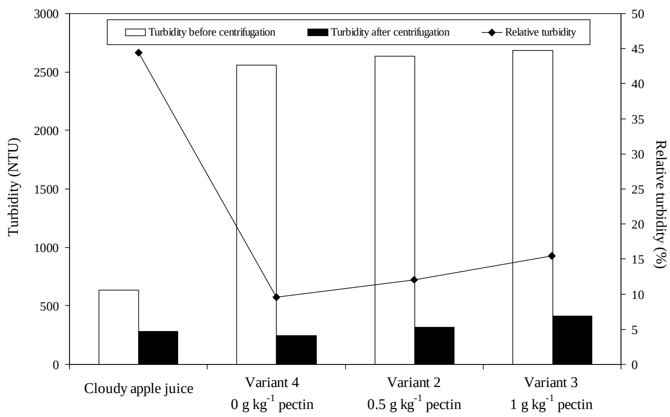

Figure 2: Turbidity and relative turbidity of cloudy apple juice and model beverages variant 2, 3 and 4 as determined by centrifugation test (4200 *g*, 15 min).

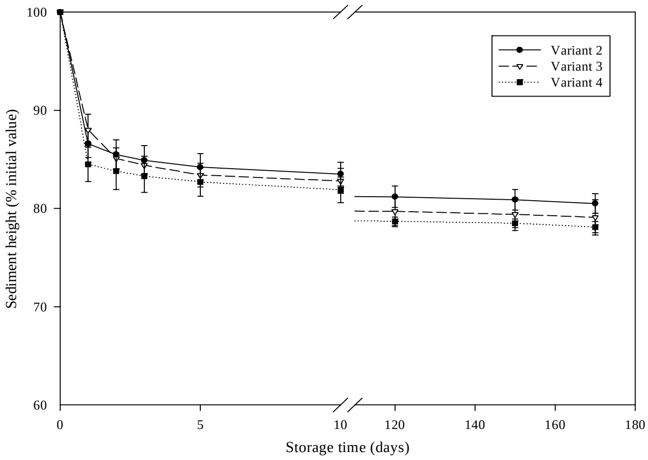

Figure 3: Sediment height of model beverages variant 2, 3 and 4 as determined by real time sedimentation test during 170 days of storage. Storage conditions: dark, about 18 °C.

Figure 4: Physical cloud stability of model beverages before (variant 2) and after 170 days (variant 4, 2 and 3) of storage (from left to right).

tion (4200 *g*, 15 min) and their corresponding relative turbidity values are shown in Fig. 2. As can be seen, the turbidity of the beverage (variant 4, no pectin added) considerably increased after blending cloudy apple juice (630 NTU) with the carrot pomace hydrolyzate, amounting to 2560 NTU. The turbidity after centrifugation remained unchanged for both beverages, resulting in lower relative turbidities. When adding 0.5 and 1 g kg^{-1} pectin, the turbidities of both beverage and serum slightly increased with a resulting increment of relative turbidity from 9.5 to 12 and 15.5 %, respectively. Hence the observed increase in turbidity upon addition of pectin might be attributed to the higher serum viscosity. By visual inspection of the sera, fine cloud particles were shown to be still suspended. As described by Reiter et al.[19], complete clarification of carrot juices even after ultra-centrifugation could not be achieved, which was ascribed to a cloud-stable particle fraction with a particle density nearly identical to the serum density. Surprisingly, despite the enzymatic degradation of the carrot pomace tissue and thus of stabilizing pectic substances, a satisfactory cloud stability of the hydrolyzate in the beverages could be achieved. According to Reiter et al.[14], carrot juices produced by enzymatic mash treatment were characterized by extreme cloud stability, suggesting that the latter is affected by proteins rather than by pectic substances.

Since previous studies revealed that the rapid centrifugation test evidently depended on the type of beverage system[17], a real time sedimentation test was additionally carried out during a period of 170 days. As evidenced in Fig. 3, within the first day after bottling a rapid sedimentation of coarse cloud particles was observed, resulting in a decline of sediment height, amounting to 13, 12 and 15 % for variants 2, 3 and 4, respectively. Throughout the remaining storage time the portion of sediment further decreased, reaching 80, 79 and 78 % for variants 2, 3 and 4, respectively. Pectin addition did not significantly affect clarification behavior of the beverages. The positive effect of pectin, however, became obvious since the beverage without pectin was characterized by a more pronounced clarification with a low turbidity value of 83 NTU after 170 days of storage, whereas beverages containing 0.5 and 1 g kg^{-1} pectin displayed improved serum turbidities of 153 and 241 NTU, respectively. In contrast to the centrifugation tests, which were carried out for evaluating the prospective long-term stabilities of the beverages over one year, supernatant turbidities of the sedimentation tests were much lower, thus demonstrating the limited applicability of the former assay to predict cloud stability of this type of beverage systems. On the other hand, Reiter et al.[19] confirmed that the centrifugation test is also suitable to separate all fast sedimenting particles with a critical size of > 10 µm from carrot juices. Nevertheless, by using both tests satisfactory cloud stability and the lack of supernatant clarification was clearly shown. Furthermore, sedimented cloud particles could easily be re-dispersed by slightly shaking the bottle. A visual comparison of the model beverages at the beginning and the end of storage is shown in Fig. 4, impressively pointing out the bright and stable orange color originating from the hydrolyzate. Depending on the process stage of acidification during carrot juice production, acidifying juices prior to pasteurization always resulted in unstable juices, whereas acidification of the coarse mash resulted in highest cloud stability[14]. Therefore, hydrolysis of carrot pomace on acidic conditions resulted in cloud stability when blending with acidic media such as apple juice.

Carotene stability: Total carotene content, expressed as the sum of α- and β-carotene, was determined after 20 weeks of storage under moderate illumination (Table 2). No significant degradation of the provitamins was observed. Variants 1 and 2 contained 12.4 and 11.7 mg L^{-1} total carotene at the beginning and 12.0 and 12.7 mg L^{-1} at the end, respectively. Even after six months under intense illumination total carotene content remained unchanged, resulting in 12.2 and 12.8 mg L^{-1} for variant 1 and 2, respectively. Moreover, formation of *cis*-isomers of β-carotene was not observed even after intense illumination. These findings support the protective role of the natural plant matrix for carotene stability, as already demonstrated for ATBC-drinks[20] and carrot juices[13,21]. The present study also shows that α- and β-carotene are extraordinarily stable even after almost complete degradation of the cell wall polymers (pectin, hemicellulose, cellulose) and confirms that the physical state of carotenes seems to be the most important factor in carotene stability. As very recently demonstrated, model preparations containing crystalline β-carotene showed pronounced stability during heating, whereas β-carotene dissolved in toluene resulted in isomerization[21]. In addition to the quantification of carotenes, color of the beverages was monitored by determination of *L*a*b** values. Ascorbic acid was added to variant 2 to avoid browning. Significant changes were not observed for either of the illumination conditions. *L*a*b** values are exemplarily given for variant 1: 31.08, 9.51 and 15.70 at the beginning *vs*. 30.33, 9.41 and 14.54 at the end of intense illumination, respectively. Addition of ascorbic acid as a water-soluble antioxidant neither affected color nor carotene content during storage. Compared to vitamin-supplemented drinks composed of carrot juice as a natural source of β-carotene, ATBC drinks containing synthetic β-carotene formulations suffered from higher isomerization rates which have been associated with nutritional consequences such as reduced provitamin A activity[13]. For the consumer the problem arises that an exact calculation of the provitamin A intake cannot be realized. Whereas in beverages based on synthetic β-carotene overages need to be added to compensate losses and guarantee the labelled content during the specified shelf life, adjustment of β-carotene content by utilization of hydrolyzed carrot pomace appears to be advantageous. So far, legislative or official guidelines concerning acceptable overage levels do not exist. The Association of German Chemists recommended that a deviation of ±30 % for provitamin A should be tolerated, however, overages of 50 % of the specified content should not be exceeded[22].

Conclusions

The results obtained in the present study demonstrate that the hydrolyzate recovered from carrot pomace may be used as a natural ingredient of functional soft drinks, as exemplified for cloudy apple juice. Thus, complete utilization of carrots was achieved by integration of carrot pomace hydrolysis into the conventional carrot juice production. Whereas carotene stability of the model beverages based on hydrolyzed carrot pomace was outstanding, cloud stability was found to be satisfactory after bottling during long-term storage. Furthermore, extended applications of the carotene-rich hydrolyzate as a coloring food, e.g. in dried products, appear to be promising. Since water-dispersible synthetic β-carotene formulations are usually based on gelatin to enhance water solubility, their application to beverages rich in polyphenolics may result in the formation of sensorically unacceptable sediments. In contrast, hydrolyzates obtained from carrot pomace represent a suitable natural alternative for the supplementation of cloudy functional drinks, especially of ATBC-drinks, since α- and β-carotene are added in their genuine proportion. Due to the almost complete degradation of the cell wall polymers, increased bioavailability of carotenes from carrots may be expected[23]. Moreover, it has been shown that the hydrolyzate also contains oligogalacturonic acids (OGAs) as a second functional ingredient, which was derived from enzymatic hydrolysis of pectic cell wall substances[10]. OGAs have recently attracted intense interest since they have been demonstrated to inhibit the adherence of bacteria to epithelial cells and might therefore be used as therapeutic agents[24,25].

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