Optimising Quality of a Mature-Green Golden Apple (*Spondias cytherea*) Drink Using Amyloglucosidase Treatment

Séverine Franquin [a,b]*, Odile Marcelin [b] and Jean-Marc Brillouet [a]

[a] Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département F LHOR, TA 50/16, 34398 Montpellier Cedex 5, France.
Phone : + 33 4 67 61 75 81 - Fax : + 33 4 67 61 44 32
Corresponding author: severinefranquin@hotmail.fr

[b] Groupe A. H. Despointes, Usine DENEL, 97132 Gros Morne, France

*Correspondence and reprints

Tel: 33(0)467615800 poste 5230; Fax: 33(0)467614433; e-mail severinefranquin@hotmail.com

Abstract

The Golden apple (*Spondias cytherea* Sonnerat or *Spondias dulcis* Forst), also called Otaheite apple or ambarella, native to Polynesia, belongs to the Anacardiaceae family, which includes the mango, *Mangifera indica* L., and the cashew, *Anacardium occidentale* L. Although this fruit is widely distributed in the Caribbean region, Asia and Central and South America, the use of mature-green fruits to make a drink is geographically restricted to the French West Indies (Martinique and Guadeloupe) and Grenada. Due to its slight acidity and astringency, and its olive green colour caused by the green pigments from the outer layers of the fruit, this drink is much appreciated by the consumers of these regions. However, in its present formulation, the initial green colour of fruits is poorly recovered and starch induces a detrimental whitish sediment in the final product. In order to improve the visual quality of the mature-green Golden apple drink, two strategies were used. First, for degrading starch and standardizing its content, the juice obtained after grinding and sieving was treated with an amyloglucosidase (AMG 300 L, Novozymes) at various concentrations (200 g/t – 700 g/t – 2 kg/t) for 15 min at 60°C after starch gelatinisation (64°C or 72°C - 15 min). Then, the treated juice was used to make nectars at 13°Brix, using a green powder prepared from Golden apple peels to enhance the colour of the nectars. These new formulations allowed the production of less sweet nectars with a green colour closer to that of the fresh fruit.

Keywords : Anacardiaceae, starch, enzymatic treatment, green pigments, nectars, colour preservation.

INTRODUCTION

The Golden apple (*Spondias cytherea* Sonnerat or *Spondias dulcis* Forst) belongs to the Anacardiaceae family which includes several important tropical fruit trees such mango (*Mangifera indica* L.) and cashew (*Anacardium occidentale* L.) (Purseglove, 1974). Native to Polynesia, it was introduced into Jamaica in 1782 and nowadays is mainly cultivated in the Caribbean region, Asia, Central America, South America and, to a lesser extent, in Africa. Golden apple is a medium-sized (15-25 m) tree with olive green hairless leaves (2-6 cm length) (Fournet, 2002). The fruit is an ellipsoid to ovoid drupe (4-12 cm length; 3-8 cm diameter) and can weigh up to 450 g (Persad, 1991; Daulmérie, 1994; Fournet, 2002). Structure of an immature green Golden apple is presented in Figure 1 and its physico-chemical characteristics are given in Table 1. When the fruit ripens, its skin and flesh turn Golden-yellow, emitting aroma components characterized in previous papers (Wong and Lai, 1995; Jirovetz et al., 1999).

The ripe fruits are used to make jams, preserves, juices (Geurts et al., 1986), and sparkling beverages (Massiot et al., 1991). Unripe Golden apple fruits are eaten in curries or green salads or made into pickles (Daulmérie, 1994), but it is mostly used to make a drink much appreciated by the consumers of the French West Indies (Martinique and Guadeloupe) and Grenada due to its slight astringency and its yellowish-green colour caused by the green pigments in the outer layers of the fruit. Briefly, fruits are washed, crushed, pressed, and the crude pulpy juice sieved prior to conditioning as a nectar (addition of sucrose and water), pasteurisation, and bottling (Marcelin, personal communication, 2003). However, this drink made with mature-green Golden apples has a high starch content, which alters the visual appearance of the drink since, on one hand, a white sediment can form in storage at the container bottom, and, on the other hand, the whiteness caused by the starch makes the olive green colour paler. Moreover, pasteurisation of the drink partially destroys the olive green colour of the nectar. Consequently, restoring this characteristic colour is achieved by adding a chlorophyll extract from corn leaves (Marcelin, personal communication, 2003).

In order to improve the visual quality of the mature-green Golden apple drink, two methods were tested. First, we used commercial amylolytic enzymes to partially or totally degrade the starch and then control its content in the final product. A previous study showed that a commercial amyloglucosidase completely hydrolysed mature-green Golden apple starch into glucose, thus increasing the total soluble solids content (Eugène, 1995). Second, we preserved the characteristic olive green colour of the fresh fruit by adding a green powder from Golden apple peels to the treated juice for making nectars at 13°Brix. The aim of this work was to investigate the feasibility of making Golden apple nectars at 13°Brix using both amylolytic enzymes after gelatinisation of the starch present in the juice and a green powder extracted from mature fruit peels as a natural colouring of nectars.

MATERIALS AND METHODS

1. Materials

1.1 Plant Material. 143 mature-green Golden apples (*Spondias cytherea* Sonnerat) harvested from several trees in domestic orchards in Martinique (French West Indies) were used as raw material.
1.2 Enzyme source. AMG 300 L from *Aspergillus niger*, a commercial amyloglucosidase preparation with 300 units enzyme per mg, was obtained from Novozymes (France).

2 Methods

2.1 Juice Extraction Process. Golden apple fruits were trimmed and washed with running water in order to remove foreign material from the skin: only intact fruits were selected. They were then crushed and pressed before sieving with vibrating sieves of 0.5 and 0.2 mm. The juice thus obtained was kept in a chamber at – 20°C until further processing.

2.2 Enzymatic Treatment. For each experiment, 10 g of sieved juice was subjected to different enzyme treatments as shown in Table 1. Preliminary experiments were carried out on the pressed and sieved juice at different temperatures of starch gelatinisation (52°C, 60°C, 65°C, 72°C, 85°C, 95°C) before using a high amyloglucosidase concentration (8 kg/t) at 52°C for 15 min (data not shown). Under these conditions, we demonstrated by graphic resolution that starch gelatinisation is optimal at 64°C and 75°C, with 97 and 100% starch degradation respectively. In a typical treatment, 10 g of fresh Golden apple juice was incubated for 15 min with stirring at 64°C or 75°C to gelatinise the starch. Then, enzyme at 200, 700 or 2 kg/t was added and incubation carried out at 60°C for 15 min. The sample was then kept in a boiling water bath for 5 min to inactivate the AMG 300 L enzyme. After cooling to room temperature, an aliquot of the treated juice (1 ml) was centrifuged (14 500 rpm for 5 min) and the supernatant used for °Brix measurement.

2.3 Preparation of Nectars. Sucrose, distilled water and a green powder extracted from Golden apple peels were mixed with juice treated with amyloglucosidase to make nectars at 13°Brix with colour preservation. After bottling in glass flasks, pasteurisation was performed in a water bath at 96°C for 4 min after heating in a microwave oven for 12 s. The glass flasks were cooled in a water bath at 4°C.

2.4 Analytical Methods. Total soluble solids (°Brix) were measured at room temperature on centrifuged juice (14 500 rpm for 5 min) using an Abbe refractometer with a measuring range of 0-32°Brix (Askar and Treptow, 1993). Starch was measured by the UV-method (Boehringer Mannheim, 1977) with correction for endogenous glucose.

RESULTS AND DISCUSSION

The effects of treating Golden apple juice with amyloglucosidase enzyme for different gelatinisation conditions and amyloglucosidase concentrations are shown in Table 2. We obtained three types of green Golden apple juices after enzyme treatment performed at 60°C for 15 minutes (with 37%, 62% and 100 % starch hydrolysis respectively). As the amyloglucosidase concentration increased, the soluble solids content varied from 6.8 to 8.0, the difference corresponding to the starch content in the raw juice without enzyme treatment (1.2 g / 100 g fresh material).

These results showed the importance of finding a balance between starch state and amyloglucosidase concentration for its optimal degradation: even if the amyloglucosidase concentration is significant, only gelatinised starch is recognized as its substrate and hydrolysed to glucose units. The liberation of glucose increased the °Brix of the juice (Table 2); thus we needed to estimate by the calculation the appropriate sucrose amount to be added to reconstitute the nectars at 13°Brix.

Using temperatures greater than 60°C affected drastically the colour of the juice and we needed to add a green powder prepared from Golden apple peels to improve the olive green colour of the reconstituted nectars. Compared to a drink commercialised in the French West Indies (Martinique and Guadeloupe), our nectars, whatever percentage of amylolysis used, had a better green colour closer to that of the fresh fruit (Figure 2).

CONCLUSION

In this study, we optimised experimental conditions (temperature of starch gelatinisation, amyloglucosidase concentration….) for partially or entirely degrading the starch. Then, we made Golden apple nectars at 13°Brix from the treated juice, using mature fruit peels to restore the green colour to that of fresh fruits. In the future, it will be possible to combine pectocellulolytic and amylolytic treatments on mature-green Golden apple fruits to both increase juice extraction yield and to degrade starch up to a specific level in order to prepare nectars at 13°Brix.

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Tables

pH	2.6 (± 0.04)
Total solids	20.4 (± 1.8)
Total soluble solids (°Brix)	10.0 (± 0.1)
Sucrose	3.1 (± 0.3)
Fructose	1.5 (± 0.2)
Glucose	1.2 (± 0.2)
Titratable acidity (g citric acid equivalents.100 g^{-1})	1.3 (± 0.03)
Citric acid	0.9 (± 0.1)
Malic acid	0.2 (± 0.02)
Oxalic acid	0.03 (± 0.01)
Proteins	0.8 (± 0.1)
Starch	7.1 (± 0.5)
Cellulose	1.1 (± 0.1)
Hemicelluloses	0.8 (± 0.2)
Lignin	0.5 (± 0.1)
Vitamin C (mg. 100 g^{-1})	52.0 (± 4.9)
Phenolic compounds (mg gallic acid equivalents.100 g^{-1})	349.5 (± 52.3)
Ashes	0.5 (± 0.06)

Table 1. Physicochemical characterization of immature green Golden apples before processing (unless otherwise stated, g.100 g^{-1} fresh material).

Gelatinisation conditions	AMG 300 L concentration (g/T)	Starch content (%)	Amylolysis (%)	Brix degree
-	0	1.21	0	6.8
64°C – 15 min	200	0.75	37	7.4
64°C – 15 min	700	0.45	62	7.4
75°C – 15 min	2000	0	100	8.0

Table 2. Starch content and Brix degree of the green Golden apple juice treated with amyloglucosidase. These conditions resulted to preliminary experiments on green Golden apple pulp (data not showed).

Figures

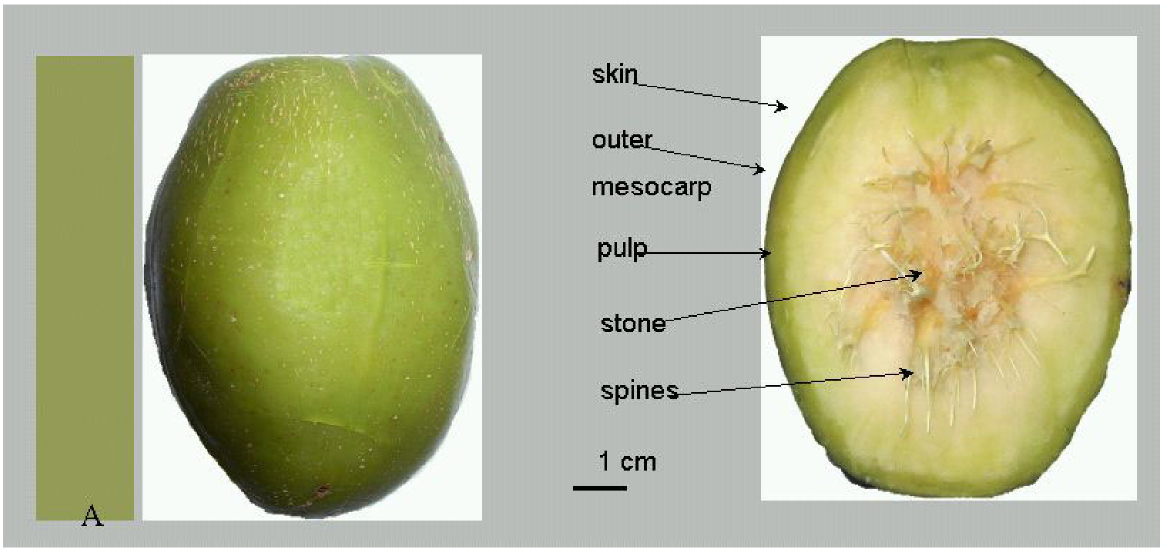

Figure 1. Structure of the fresh immature green Golden apple. (**A**) skin colour reconstituted from measured (*L*, *a*, *b*) parameters (L = + 51.7 ± 1.7; a = - 12.2 ± 1.7; b = + + 36.0 ± 1.8).

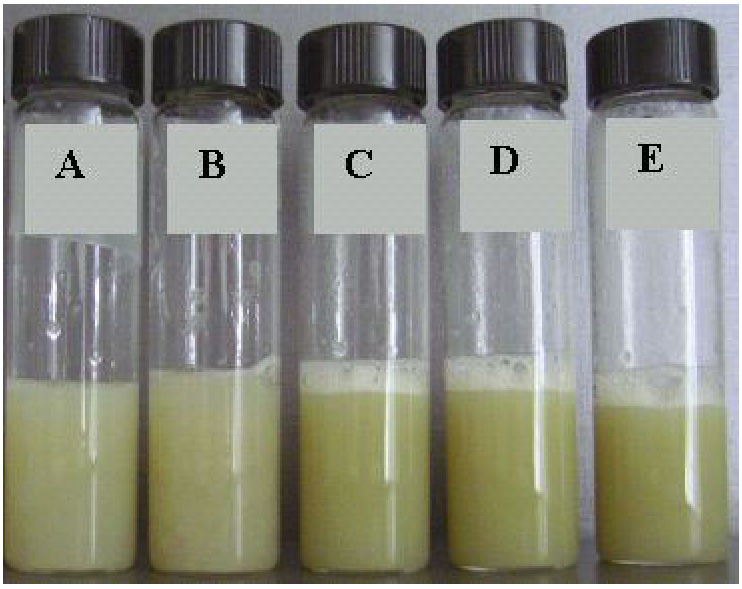

Figure 2. Green Golden apple nectars with different starch contents and colour restoration using peel powder. Legend: commercial Golden apple nectars (A). Nectars with peel powder and 0 (B), 37 (C), 62 (D), 100% (E) starch hydrolysis after amyloglucosidase treatment (with enzyme concentration of 200 g/t; 700 g/t and 2 kg/t respectively) performed at 60°C for 15 minutes.