ELSEVIER

Carbohydrate Polymers 38 (1999) 179–182

Carbohydrate Polymers

# Natural colloidal particles: the mechanism of the specific interaction between hesperidin and pectin

N. Ben-Shalom, R. Pinto

*Department of Food Science, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel*

Received 30 July 1997; received in revised form 5 January 1998; accepted 20 January 1998

**Abstract**

Hesperidin and pectin can form stable colloidal particles in solution. The specific interaction between hesperidin and pectin was shown to be due to the neutral sugars (NS) in the hesperidin molecule and in the polyuronide polymer. Two types of pectin were identified, both of which formed colloidal particles in the solution. One type formed stable particles, the other formed particles that flocculated. Less than 4% of the pectin formed stable particles with the hesperidin, and most of the commercial pectin did not interact with it. The specific fraction of the pectin, which formed the stable particles, contained about 80% neutral sugars (NS) and more than 10% of rhamnose. The specific fraction which formed the unstable particles contained about 40% NS and about 2% rhamnose. Good correlation was found between the neutral sugar content of the pectin and the stabilization of the hesperidin in the solution. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* Hesperidin; Pectin; Neutral sugars; Interaction

## 1. Introduction

Cloud from orange juice contains mainly pectins, proteins and lipids (Baker and Bruemmer, 1969). It also contains hesperidin in a crystallized form and contributes to its flocculation (Baker and Bruemmer, 1972). Hesperidin seems to be located in a soluble form in the vacuole of the intact cell and to crystallize out of the cell after membrane damage (Bennett and Alback, 1981). An increase in the turbidity of the fresh orange serum has been shown to be connected with hesperidin crystal formation (Mizrahi and Berk, 1970).

Cloud formation between pectin and hesperidin in our model system is a result of interaction between the polysaccharide polymer and the insoluble flavonoid (Ben-Shalom et al., 1984). The stability of this cloud is a function of the ratio between the amount of the flavonoid and the molecular weight of the polymer (Ben-Shalom et al., 1984). With pectin, hesperidin forms a particle, which could be isolated by sucrose gradient centrifugation (Ben-Shalom et al., 1985). The pectin–hesperidin particle has been shown to be a good model for studying the flocculation of the juice cloud by calcium binding (Ben-Shalom et al., 1985).

The aim of the present work is to show the specific interaction between hesperidin and pectin and to suggest a model for the type of pectin, which can stabilize the hesperidin particles in the aqueous solution.

## 2. Materials and methods

Pectin of type 1 and type 2 from citrus fruit (Sigma), with 80 and 82% degree of esterification (Klavons and Bennett, 1986) and molecular weight of 82 000 and 85 000 Da (Christensen, 1954) was used. The pectin, citric acid, tri–potassium–citrate, sucrose, sodium benzoate, hesperidin, ethanol and dimethylformamide were purchased from Sigma Chemical Co., St. Louis, MO, USA.

The model cloud system contains a solution of citric acid (1%) tri–potassium citrate (0.67%), sodium benzoate (0.1%) and pectin (0.2%) at pH 3.8, as described previously (Ben-Shalom et al., 1985). To form the colloidal particles, hesperidin (10 mg/ml) was solubilized in 0.2 N NaOH and added to the above pectin solution. Stability was determined by measuring turbidity at 660 nm. Separation of the hesperidin–pectin particles on sucrose gradient was performed with 60 and 70% sucrose. The hesperidin–pectin particles were dissolved in 40% sucrose and put over 60% sucrose. Separation of the particles was done with a Beckman ultracentrifuge at 2 000 g for 30 min, as described previously (Ben-Shalom et al., 1985).

The stable particles formed from pectin type 1; which separated on the sucrose gradient; and the flocculated particles obtained from pectin type 2; after the formation of unstable particles between the hesperidin and the pectin; were further purified by repeated washing with 70% ethanol


PII: S0144-8617(98)00111-8

Figure 1. Addition of 0.015% hesperidin solution, previously solubilized in NaOH, to: 1) 0.2% commercial pectin, of which the particles flocculate on the bottom of the tube; 2) 0.2% commercial pectin, of which formed stable colloidal particles in the solution.

and then dimethylformamide in order to remove any excess sucrose and hesperidin, and thus obtain the purified pectin. This was finally dried with 100% ethanol. Neutral sugars were removed by hydrolysis with trifluoroacetic acid, acetylated and analyzed by GC according to Blakeney et al., (1983).

All statistical analysis is based on four replications (means, standard deviation of means, analysis of variance), and was performed using SAS package (SAS Institute., 1986).

## 3. Results and discussion

Stable natural colloidal particles (cloud) can be formed as a result of a specific interaction between hesperidin and pectin. This interaction was repeated in our laboratory many times, although we are aware that at least one group, in Pasadena, CA, was unsuccessful (Klavons and Bennett, 1987).

Addition of hesperidin (previously solubilized in NaOH) to potassium citrate buffer, caused the flavonoid to precipitate completely on the bottom of the tube, with the formation of large crystals that could be observed microscopically. The addition of hesperidin (0.015%) to a commercial pectin solution (0.2%) initiated the formation of stable colloidal particles (cloud) in the solution (Ben-Shalom et al., 1984; Ben-Shalom et al., 1984). This experiment showed us that we could form a stable cloud in aqueous solution using natural orange ingredients.

In a previous paper (Ben-Shalom et al., 1984), it was reported that the molecular weight of the pectin had an important effect on the stability of the hesperidin–pectin particles; decreasing the size of the pectin polymer decreased the stability of the particles. It was also found that changing the degree of esterification of the pectin, did not affect its interaction with the hesperidin and the intensity of colloidal particle formation (Ben-Shalom et al., 1985).

A study was made on the possibility that the neutral sugars (which are an important part of the pectin polymer) are involved in the specific interaction between hesperidin and pectin. The aglycone of the hesperidin (hesperetin) was separated from its sugar moiety (rhamnose and glucose) by acid hydrolysis and mixed with the pectin. Colloidal particles were not formed, regardless of the ratio between the hesperetin and the pectin. This showed that the recognition site of the hesperidin molecule for the pectin was located in the sugar moiety, and the hydrolysis caused a complete loss of the hesperidin's ability to interact with the pectin.

In order to show the role of the neutral sugars (NS) in the recognition of the hesperidin by the pectin, two different commercial pectins (Sigma) were identified, of which one formed stable colloidal particles in the solution and the other did not (Fig. 1), although their molecular weights and their degrees of esterification were found to be almost identical. The particles of the stable pectin were separated on a sucrose gradient (Ben-Shalom et al., 1985) and the precipitate of the unstable particles was collected. The two differentiated pectins were further purified to remove their excess sugar and hesperidin, by repeated washing with 70% ethanol and dimethylformamide. Both pectins, which reacted with the hesperidin comprised less than 4% of the total soluble pectin which, was initially mixed with it.

Analysis of the composition of the two differentiated pectins showed that these two fractions were very rich in NS, as compared with the commercial pectin (Table 1). The

Table 1
Percentages of neutral sugars (NS) and galacturonic acid (GA) from the pectins

| Type of sugar | Commercial pectin Type 1 | Stable pectin fraction Type 1 | Commercial pectin Type II | precipitate pectin fraction Type II |
|---|---|---|---|---|
| % N.S. and G.A from the pectin | | | | |
| Total Neutral Sugars | 12.9 + 1.4 | 83.1 + 6.1 | 10.2 + 1.4 | 40.85 + 2.9 |
| Galacturonic Acid | 87.1 + 5.8 | 16.9 + 1.5 | 89.8 + 4.9 | 59.15 + 3.1 |

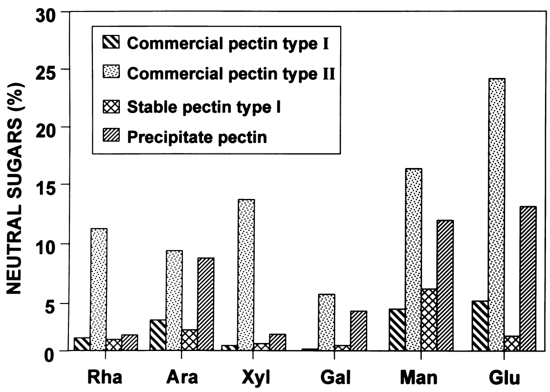


Figure 2. Composition of the neutral sugars in four different pectins. 1) Commercial pectin type I. 2) The pectin fraction type I which formed the stable particles. 3) Commercial pectin type II. 4) Pectin type II, which formed unstable hesperidin-pectin particles. Rha. rhamnose; Ara. arabinose; Xyl. xylose; Ma. Mannose; Gal. galactose; Glu. glucose.

type 1 pectin fraction, which stabilized the hesperidin in the solution contained more that 80% NS, whereas the pectin fraction type 2, which reacted with the pectin, but precipitated with it, contained about 40% NS. Both specific pectins (types 1 and 2) had an extremely high NS content, as compared with average of 10% in the commercial pectins (Table 1). The contents of all the various neutral sugars increased in pectin fractions type 1 and type 2. The main changes in pectin type 1 were in rhamnose, xylose, mannose and glucose, those in pectin type 2 were in glucose, mannose, xylose and arabinose. The main differences in NS between the two pectin fractions were in the high contents of xylose and rhamnose in the type 1 fraction (Fig. 2).

It was found that interaction of the hesperidin with several other polysaccharides, such as guar gum, locust bean gum, carrageenan, gum arabic and xanthan, initiated the formation of turbidity, but resulted in flocculation of the particles and the formation of a clear solution. Apart from pectin type 1, we found no other polysaccharide able to stabilize hesperidin in the solution.

To check the possibility that the specific NS in the pectin are important for stabilization of the colloidal particles in the solution, stable colloidal particles, which had previously been formed from 0.2% pectin and 0.015% hesperidin, were separated on a sucrose gradient. The hesperidin–pectin particles moved through the gradient, but the low density soluble pectin (which did not react with the hesperidin and which was more than 96% of the pectin) was left above the gradient. Mixing the hesperidin with this new fraction of the soluble pectin in concentrations of 0.2%–0.8%, resulted in a relatively low yield of particle formation, which ended with flocculation. Mixing the hesperidin with the commercial pectin type 2 (the one which tended to flocculate), in a concentration twice that normally used, (0.4%), did not stabilize the hesperidin in the solution. It was found that, although the total amount of neutral sugars in the above two pectins was equal to or greater than that in pectin type 1 fraction, the pectin 2 with 80% NS was the only one which could stabilize the hesperidin in the solution. This means that the amount of the NS in the pectin polymer is more important than its total amount in the solution.

Under normal conditions, hesperidin molecules are almost insoluble in aqueous solution (15 mg/l), and tend to crystallize quickly. The size of the crystals increases to a certain critical point, at which they precipitate.

We assume that the differences in the stabilization of the hesperidin by the three types of pectin (the commercial one, the 40% and the 80%), is due to the specific interaction of the NS of the pectin with the glycoside residue of the hesperidin by hydrogen bonds.

Based on the above assumptions, a polymer with the highest content of NS branches can interact with the hesperidin much more strongly and tightly than one with low content of NS. As a result of this interaction, the particles which formed between the hesperidin and pectin are stable in the aqueous solution.

In the second type, the one with 40% NS,the pectin is probably binding to the hesperidin much less tightly and the particles tend to flocculate. Commercial pectins which have only a low NS content, will hardly interact at all, with the hesperidin molecules.

One of the main conclusions from our work, is that commercial pectin contains several types of pectin, each of which interacts with hesperidin in a different way.

This work may also help in understanding the cloud of the orange juice. It can explain some phenomena in the orange juice which were difficult to understand before. It is possible that in the orange juice, as in our model, there exists at least three different types of pectin. One type is found in the clear supernatant of the juice and has almost no interaction with the hesperidin. Another type forms part of the stable colloidal particles in the juice, by interacting with the hesperidin. The third type is the one, which interacts with the hesperidin, but does not form stable particles with it. It later flocculates and appears as part of the precipitate.

## Acknowledgements

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel No. 1702-Em 1997 series.

## References

Baker, R. A., & Bruemmer, J. H. (1969). Cloud stability in the absence of orange juice soluble constituents. *Proc. Fla. State Hortic. Soc.*, *82*, 217–220.

Baker, R. A., & Bruemmer, J. H. (1972). Influence of pectate-hesperidin floc on orange juice clarification. *Proc. Fla. State Hort. Soc.*, *85*, 225–229.

Bennett, D. R., & Albach, F. R. (1981). The nature of freeze induced white spots on orange segment walls. *J. Agric. Food Chem.*, *29*, 511–514.

Ben-Shalom, N., Shomer, I., & Kanner, J. (1984). Pectin–hesperidin interaction in a citrus cloud model system; The effect of pectin degradation. *Lebensm-Wiss. u.-Technol.*, *17*, 125–128.

Ben-Shalom, N., Pinto, R., Kanner, J., & Berman, M. (1985 > ). A model system of natural orange juice cloud: effect of calcium on hesperidin–pectin particles. *J. Food Sci.*, *50*, 1130–1132.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). Simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.*, *113*, 291–299.

Christensen, P. W. (1954). Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. *J. Food Res.*, *19*, 163–166.

Klavons, A. J., & Bennett, R. D. (1987). Determination of methanol using alcohol oxidase and its application to methyl ester content of pectins. *J. Agric. Food Chem.*, *35*, 159–161.

Mizrahi, S., & Berk, Z. (1970). Physico–chemical characteristics of orange juice cloud. *J. Sci. Food Agric.*, *21*, 250–254.