DELAY OF RUTIN CRYSTAL FORMATION IN ASPARAGUS PICKLES

Retraso de la Formación de Cristales de Rutina en Encurtidos de Espárragos

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SUMMARY

Rutin (quercetin -3-0- rhamnoglucoside) crystallization on pickled asparagus spears has been studied, but it is unknown the prevention of this phenomena. The objective of this research was to prevent, or retard rutin crystallization in asparagus pickles by using gums. Asparagus was pickled with brines containing pectin and xanthan, one compound for brine. The asparagus treated with xanthan were first soaked in sodium bicarbonate 0.2 M and then processed. During pickles storage, rutin concentration was determined in brines and tissues at 1 day and then monthly. Rutin determination was made by HPLC. Titrable acidity and pH were also monitored to ensure the pickles stability. The times for crystals appearance were visually monitored. Control samples were made as a reference. In each group of samples, significant differences were observed between rutin content in the brine and tissue during storage, but these differences did not account in the prevention of rutin crystal formation. Rutin crystal formation was slightly delayed in the samples treated with pectin but not prevented.

Keywords: rutin, Crystallization, desupersaturation, xanthan, pectin, asparagus.

RESUMEN

La cristalización de la rutina (quercetina -3-0- ramnoglucosido) sobre espárragos encurtidos ha sido estudiada, pero no se ha logrado prevenir este fenómeno. El objetivo de esta investigación fue prevenir o retardar la cristalización de la rutina en encurtidos de espárragos, mediante el uso de gomas. Lotes de espárragos fueron encurtidos con salmueras a las cuales se les añadió pectina y xantano, una goma por cada lote de espárragos. Los espárragos tratados con xantano fueron lavados previamente con una solución de bicarbonato de sodio 0.2 M. Durante el almacenamiento de los espárragos encurtidos se determinó la concentración de rutina mediante HPLC. La acidez titulable y el pH fueron monitoreados durante el almacenamiento para determinar la estabilidad de los espárragos encurtidos. Se monitoreó visualmente la aparición de los cristales de rutina y en todos los casos se prepararon muestras control sin ningún tipo de tratamiento. En cada grupo de muestras se encontraron, durante el almacenamiento, diferencias significativas entre el contenido de rutina en la salmuera y en los tejidos de los espárragos encurtidos, pero estas diferencias no son suficientemente significativas como para prevenir la formación de los cristales de rutina. Se retardó ligeramente la cristalización de la rutina en las muestras tratadas con pectina, pero la cristalización no se previno de manera absoluta.

Palabras clave: rutina, Cristalización, desobresaturación, xantano, pectina, espárragos.

INTRODUCTION

Asparagus (*Asparagus officinalis*) is a perishable crop that can be preserved as frozen, canned, or pickled. When asparagus is pickled, an undesirable precipitate appears on its surface, producing rejection from the consumers. The precipitate in asparagus pickles was first identified by Campbell (1939) as a flavonol glucoside, and later DeEds and Couch (1947) and Fuleki (1999) identified asparagus pickles crystals as the flavonol rutin (quercetin -3-0- rhamnoglucoside). Although rutin crystals are innocuous for the human being, their undesirable appearance on the pickled asparagus produces the perception of a spoiled product in consumers. Therefore, the idea is to find effective methods to inhibit, retard, or minimize rutin crystal formation in asparagus pickles.

The hypotheses of previous researchers suggest some ways to attempt or reduce rutin crystal formation. Fuleki (1999) pointed out that minimizing the rutin concentration in asparagus could prevent the rutin crystallization. Furthermore, this author also attributed the crystal formation to the low pH in pickled asparagus, but pH of asparagus pickled is difficult to modify because it is a low acidity product.

The addition of gums, a method typically used in food systems to prevent crystal formation, can be assayed. Phillips and Williams (2000) reported the use of pectins and some other hydrocolloids in preventing crystal formation in ice cream and sugar. Adapa and others (2000) reported xanthan as one of the gums used to prevent crystallization in ice creams. The use of gums in preventing crystal formation is attributed to the reduction in the molecular mobility (Hartel 2001).

Rutin crystals are located on the surface area of asparagus spears. Therefore, one way to retard rutin crystal formation can be by washing the asparagus with an alkaline solution like sodium bicarbonate in which rutin is soluble. Windholz and others (1976) reported rutin as a compound soluble at alkaline pH.

Based on the previous information the objective of this research was to prevent, or retard rutin crystal formation in asparagus pickles, by using gums.

MATERIALS AND METHODS

Materials

Methanol (MeOH) and High Performance Liquid Chromatographic (HPLC) grade acetonitrile (MeCN) were obtained from EMD Chemicals Inc (Gibbstown, NJ). Trifluoroacetic (TFA) HPLC grade was obtained from J.T Backer (Phillipsburg, NJ. USA). Sodium bicarbonate was USP grade reagents. The

sodium chloride salt was food grade, and the vinegar was commercial 5% acetic acid. Xanthan and pectins were obtained from Danisco (New Cetury, KS).

Sources of asparagus and processing

Green asparagus spears were obtained form the local market (Fayetteville, Arkansas USA), and immediately processed as pickles in glass jars. Asparagus spears were washed in tap water, cut 15 cm from the tips, blanched in hot water at 88 °C for 3 min with the tips up, cooled by spraying tap water at 20 °C, and drained. Asparagus was put into jars (11 x 8 cm) with a fill ratio of 50:50 (220 ± 10 g asparagus: 210 ± 10 mL of brine). Then the cover brine was added, and the jars were sealed. The cover brine was 20 % commercial acetic acid, 6 % of pickling salt, and the gums assayed to prevent or retard rutin crystal formation. This brine was called modified brine. Pasteurization was applied by submerging the jars in boiling water (Equatherm water bath, model 299-729. Lab Line Instruments. Inc. Dubuque, IA) until the temperature in the cold point reached 74 °C. The temperature was determined with a thermocouple (Doris 400 series from VAS Engineering. San Diego, CA. US). The temperature was decreased by sprinkling with tap water at 20 °C.

Samples were stored at 25 °C. Analyses were made at 7 d (considered time 0) to ensure equilibration, and monthly until crystal appeared. In each group of asparagus processed, a group of asparagus was pickled with brine containing no gums, as control.

Treatments and pretreatment

a. **Pectin Treatment.** This treatment was applied by adding 0.5% pectin in the brine (modified brine).
b. **The xanthan treatment.** For this treatment the asparagus were soaked for 3 min in a 0.2 M sodium bicarbonate solution before processing. The brines were modified by adding 0.1%, 0.2%, and 0.3% xanthan.

Rutin content

The rutin content was determined in the treated asparagus pickles and in the control samples. This procedure was carried out in order to determine whether the treatments produced differences in the rutin content in the brine or in the tissue that can be significant in the prevention of rutin crystal formation. Rutin content was determined by the general analysis procedure described by Makris and Rossiter (2001) with slight modifications as follow. In the jars, the brine was separated from the asparagus tissue. For the rutin content in asparagus tissue, the asparagus were finely chopped and homogenized with a Brinkman homogenizer (Brinkman Instruments, INC. Westbury, NY. USA).

Approximately 25 g of asparagus from each jar were weighed and extracted three times with 80 mL of 80% aqueous MeOH. The first two extractions were made by homogenizing and the remaining extraction by mixing for 5 min with a magnetic stirrer. The three extracts were collected and then filtered under a vacuum system (Alltech vacuum system coupled with a vacuum pump Millipore Waters Milford, MA. US) with Whatman # 4 filter paper.

Extracts were placed in a round flask with 0.5 mL of 30% trifluoroacetic acid (TFA). The extracts with volumes about 240 mL were concentrated to 30 or 50 mL in a rotary evaporator (Brinkmann Instruments, INC. Westbury, NY), then extracted in a separatory funnel with 20 mL chloroform three times. The final volume was made to 200 or 250 mL with MeOH in a volumetric flask. The solution was filtered with a 0.45 µm syringe filter and analyzed by High Performance Liquid Chromatography (HPLC) with a Waters chromatography system fitted with a Symmetry C_{18} 5 µm, 3.9 x 150 mm column and UV detector (Waters Milford, MA). The eluents were as follows: "A" was aqueous TFA at pH 2.3 and "B" MeCN/ aqueous TFA in 6/4 proportions. I used 70% to 60% "A" for 10 min while "B" was 30% to 40%, 60% to 70% "A" for 2 min while "B" was 40% to 30%, and then isocratic for 5 min. Flow rate of 1 mL/min was used. The analysis of the brines was performed in the same chromatographic system after dissolving them with MeOH, filtering through the Whatman # 1 filter paper and through a 0.45 µm syringe filter.

Titrable acidity and pH

For all the treatments pH and titrable acidity were evaluated, because variations in the pH may influence the rutin crystal formation, and also because these parameters could give an idea about the stability of the samples. I used the method described by Buescher and Burgin (1988) for titrable acidity in the brines. The pH measurements were done in a portable IQ200 pH meter from Scientific Instruments, Inc (Barrington, IL. US), with a stainless steel probe including ISFET sensor.

Statistical analysis

Three replications of each treatment were made. Therefore, each value represents the mean of the triplicate analyses. Statistical analysis was performed by JMP IN version 5.1 (SAS Institute, INC).

RESULTS AND DISCUSION

The pH (3.70 ± 0.02) and titrable acidity (0.81 ± 0.03) of all the samples showed that the samples did not change significantly ($p < 0.05$) after equilibration time; thus, all the samples were considered stable.

In the treatments with xanthan and pectin some significant differences ($p < 0.05$) were found in the rutin concentration between the treatments at different times (Fig. 1 and 2). These statistical differences did not have significant impact in preventing rutin crystal formation.

Samples pretreated with a sodium bicarbonate solution and with different amounts of xanthan (Fig. 1) and the treatment with pectin (Fig. 2) showed the same pattern of rutin migration from the tissues into the brine, in which the rutin concentration increased from the equilibration time to the first month and then decreased in a desupersaturation process. In the xanthan treatment, the brine with highest rutin content at the 7 d was the control. In general the crystallization was faster in samples treated with xanthan in which all the asparagus had crystals at the end of equilibration time (7 d). Because of the high viscosity of the samples with xanthan, it was difficult to get uniform samples for the brine analysis. Many gas bubbles were observed in the jars and persisted through out this time.

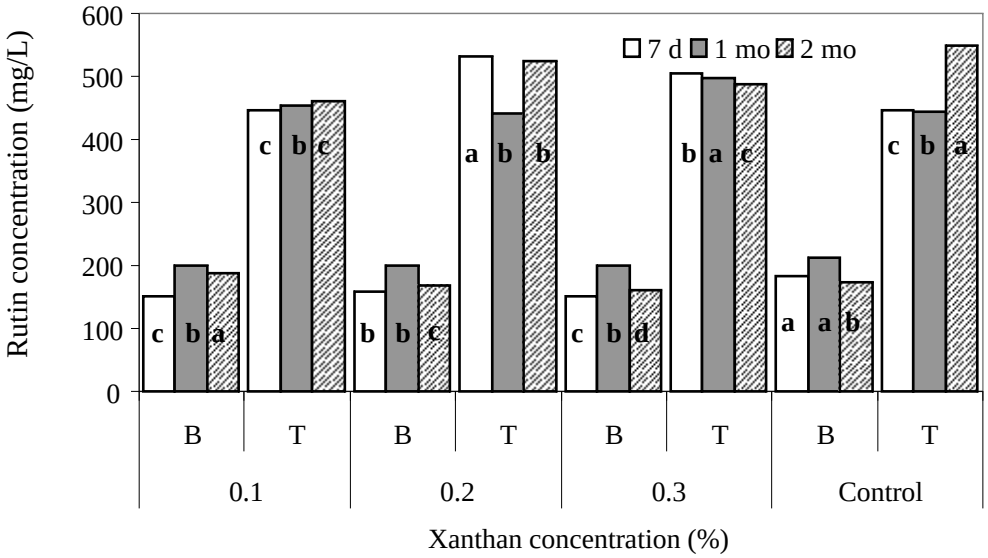

Fig. 1 Changes in the rutin concentration in brines (B) and tissues (T) of asparagus pickled pretreated by soaking the asparagus 3 min with a 0.2 M sodium bicarbonate solution and with different amounts of xanthan in the cover brine. Samples of brines or tissues with the same letter and colors bars were not significantly different ($p<0.05$)

In samples with pectin the rutin crystallization was delayed as follows. Thirty percent of the jars formed crystals at 7d, 60% at 1 mo, and 100% at 2 mo, while, in the control, all the asparagus samples formed crystals at 7 d. In general, thickeners reduce the crystallization due to an increase in the bulk viscosity. This reduction in the crystallization is related to compounds in solution (Hartel, 2001).

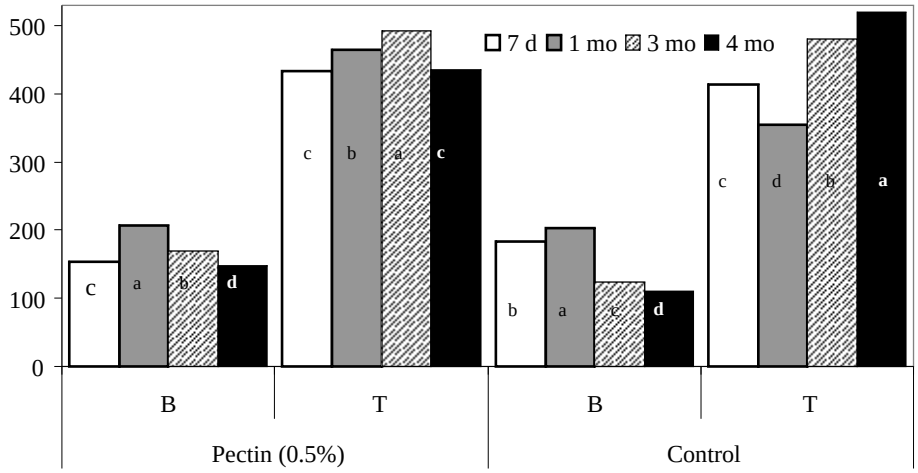

Fig. 2 Changes in the rutin concentration in brines (B) and tissues (T) of asparagus pickled with and without pectin in the cover brine. Samples of brines or tissues with the same letter and color bars were not significantly different ($p<0.05$)

All these treatments were based on the hypothesis sustained by Campbell (1939) and Hernandez and Vosti (1963) of rutin migration into the brine during the thermal treatment and brine desupersaturation with subsequent crystallization when the brines cool down. However, we observed that rutin concentration increased in the brine during the first month and desupersaturated after that time. It indicates that not all the rutin was released from the tissue during the thermal treatment; instead rutin migrated progressively into the brine after the pasteurization when stored. This implies that the brine solution was not saturated when the crystallization started. Therefore, in pickled asparagus, part of the rutin got solubilized in the brine and some stayed attached to the brine in a crystalline form.

The rutin solubility in all the asparagus pickles brine at 1 mo (pH 3.7) was higher (more than 200 mg/L) than the solubility found by Hernandez and Vosti (1963) at the same pH in acidified water solutions, the solubility found by Lueck (1970) at the equilibrium in buffer acetate at pH 8, and the rutin solubility found by Barreto and Buescher (2004) at the equilibrium in buffer acetate pH 3.7. This difference in the solubility could be due to changes in the characteristics of the solution produced when different components migrate into the brine during the equilibration time. Some of the components that can migrate into the brine are minerals (Zurera-Cosano and Moreno-Rojas 1990), which increased the rutin solubility (Griffith and others (1955). Other substances released during pasteurization and equilibration are pectins.

The maximum rutin concentration in the brine of pickled asparagus at 1 mo and the further desupersaturation in all the samples were similar to the desupersaturation process in acetate buffer reported by Lueck (1970) and Barreto and Buescher (2004).

CONCLUSIONS

Rutin crystal formation in pickled asparagus was not definitively prevented for the pretreatment with sodium bicarbonate solution or the treatment wit xanthan or pectin. However, rutin crystallization was slightly delayed in samples with 0.5% pectin in the modified cover brine, but it can not be used as a definitive treatment in preventing rutin crystal formation in asparagus pickles. The amount of rutin that migrated into the pickled asparagus brine during the pasteurization was very low, but rutin concentration increased in the pickled brine during the first month after which rutin concentration in the brine decreased and probably helped to increase the rutin crystal formation during the brine desupersaturation latter observed.

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