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Effects of Pasteurization on Antihyperglycemic and Chemical Parameter of Xoconostle (*Stenocereus stellatus*) Juice

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Abstract

Background and Objective: The antihyperglycemic effect is associated with the pre-hispanic fruit xoconostle or tunillo (*Stenocereus stellatus*, Pfeiffer and Riccobono). This fruit includes in various varieties, distinguished by color. Xoconostle fruits are highly perishable. Therefore, the aim of this study was to assess antihyperglycemic effects of xoconostle juice before (fresh) and after pasteurization. The study focused on the white and red varieties of xoconostle.

Material and Methods: In this study, the method involved collecting juice from xoconostle fruits, followed by pasteurization. Chemical, physical and microbial parameters were assessed for the juice and the ability to decrease capillary glucose levels (antihyperglycemic effect) was assessed in male Wistar rats.

Results and Conclusion: Pasteurization process led to decreases in total phenolic content of the red variety of xoconostle fruit, while the white variety showed increases in malic acid content. Despite these changes, fresh and pasteurized juices of the two varieties showed lower blood glucose levels, compared to the control group. Red variety demonstrated a stronger antihyperglycemic effect. In conclusion, pasteurization did not affect pharmacological effects of xoconostle juice, making it a viable preservation method without compromising the antihyperglycemic characteristics. Results of this research suggest a conservation method which preserve the antihyperglycemic effects while extending its shelf life.

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1. Introduction

Diabetes mellitus is a significant chronic disease that primarily affects humans. It is characterized by high blood sugar levels (hyperglycemia), resulting from deficiencies in insulin secretion or action or the two phenomena. Numerous studies have demonstrated that diets rich in fruits and vegetables can include significant effects on glucose levels in individuals with diabetes [1-5]. Polyphenols in tropical fruits such as orange (*Citrus sinensis*), caimito (*Chrysophyllum cainito*), annona (*Annona squamosa*), mango (*Mangifera indica*) and vegetables contribute to various sensory qualities, including astringency, color and flavor [6,7]. These characteristics are not only detected in the pulp but also detected in the peel. However this is considered as waste [7]. Additionally, they include health-promoting characteristics, including prevention of cardiovascular diseases (CVDs). It has been observed that catechins and specifically isoflavones can enhance muscle vascular function and decrease peripheral insulin resistance [8]. Compounds e.g. resveratrol have been shown to affect functions of B pancreatic cells; thereby, decreasing blood glucose levels. Additionally, they exert anti-inflammatory and antioxidant effects [9,10]. These types of foods that include the ability to improve health are called functional foods. However, the question is that if they preserve their characteristics after thermal processing. Nouri and Abbasi reported changes in the content of total phenols associated with various forms of processing [11] while the total phenols have frequently been associated with antioxidant effects [6] with medical importance [12].

In Mexico, a fruit called tunillo or sweet xoconostle is grown. It is addressed as a functional food that can provide protections against chronic diseases such as diabetes due to its high contents of total polyphenols, betalains and organic acids such as ascorbic acid [13]. Functional characteristics of this fruit are closely associated with its antioxidant capacity, which helps decreasing presence of free radicals in the body [14], similar to other funtional foods such as lychee [15] and annona [16-18]. Sweet xoconostle or tunillo is a fruit of *Stenocereus stellatus*, found in the Mexican states of Oaxaca and Puebla. These plants grow to heights of 2-4 m and include stems with 8-12 "ribs" and more spines than the May pitayos (*S. pruinosus*). The average weight of the fruit is 65-150 g and the pulp varies in color, ranging from red, yellow and white to purple with a bittersweet flavor. The Mixtec name for the sweet xoconostle differs slightly between the localities and is referred to as tnu dichi or too dichi. These names signify cactus or stick that can produce firewood (tutnu) and bears fruits. As previously stated, fruits of *S. stellatus* are cultivated and marketed in Mixteca of Oaxaca and Valle de Tehuacan [19]. However, studies on the development of products with tunillo fruit are limited with the production of jam as the only use at the present. Hence, the aim of this study was to investigate antihyperglycemic and chemical changes in pasteurized juices from *S. stellatus* fruits as pasteurization extends the shelf life of fresh juices without losing their antihyperglycemic effects.

2. Materials and Methods

2.1 Biological materials

In total, 39 white fruits and 33 red fruits of sweet xoconostle were used in this study. Red and white varieties were purchased in September 2017, San Juan Joluxtla, Cosoltepec Municipality, Oaxaca State, Mexico.

2.2 Preparation of sweet xoconostle juice

Fruit samples were first weighed to calculate the yield, which was reported based on the fresh weight. These were washed and disinfected using chlorinated water. Then, fruits were peeled manually and cut into iregular small pieces, which were further processed using fruit extractor (Turmix, Mexico) to separate bagasse from the juice. The total juice collected was divided into two batches. One of the batches was lyophilized directly via vacuum dehydration using Labconco Model 6 lyophilizer (Labconco, USA). The other batch was pasteurized before direct lyophilization via vacuum dehydration using Labconco Model 6 lyophilizer (Labconco, USA). The two types of samples were stored at -20 °C for further analysis [20].

2.3 Pasteurization of the juice

To pasteurize juice samples, a low temperature holding process was used acoording with the report by Gomez-Covarrubias et al 2020 [21]. The pasteurized samples were immediately cooled down on ice for 15 min [21]. An aliquot of the pasteurized juices was stored at -20 °C for further chemical analyses, the other part was lyophilized through vacuum dehydration using Labconco Model 6 lyophilizer (Labconco, USA) for pharmacological analysis.

2.4. Total coliform count

Dilutions of 10^1, 10^3 and 10^5 in 1% peptone (Merck, USA) in distilled water (w v^{-1}) were prepared to 1 ml of each juice was achieved. Then, 5 µl of each dilution were inoculated to Luria-Betani agar (BD Difco, USA) using plate counting method. Plates were incubated at 37 °C for 48 h and then total coliform count was carried out [22].

2.5 Chemical analysis of the juice

Briefly, pH of 1:10 diluted juice was measured using OAKTON pH700 pH meter (OAKTON pH700, Cole-Parmer, USA). Titrable acidity was expressed as a malic acid percentage [21]. The total soluble solid as degrees Brix (°Bx) was assessed in fresh and pasteurized juice with a manual refractometer (Pal-1 pocket refract-ometer, Atago, Japan) [21]. The total phenolic content of compounds was assessed using Folin-Ciocalteu reagent adapted by this research group [12] by measuring absorbance at 765 nm using JENWAY 6705 UV/VIS spectrometer (Cole-Parmer, UK). Flavonoids were assessed using colorimetric method of aluminum chloride at 415 nm and JENWAY 6705 UV/VIS spectrometer (Cole-Parmer, UK) [23]. To analyze the compounds, an aliquot of 0.1 ml of juice was collected from each sample, including the lyophilized juice of sweet xoconostle as well as the pasteurized and fresh juices. The aliquots were dissolved in distilled water and sample (1 ml) was collected in triplicate for the betacyanin and betaxanthine analyses using spectrophotometric method based on Cervantes et al. [14].

2.6 *In vivo* antihyperglycemic activity of the juices

To assess antihyperglycemic activity of the sweet xoconostle or tunillo juice, male Wistar rats aged 21 d and weighed 145-180 g were used. These rats were purchased from the Bioterium of University UAM-Iztapalapa and handled based on the regulations by the Institutional Committee for Care and Use of Laboratory Animals, including the Official Mexican Standard (NOM-062-ZOO-1999). The oral glucose load model was used to physiologically induce diabetes mellitus [10]. To plot the glucose tolerance test curve, rats were administered a glucose dose of 2 g kg^{-1} orally and the blood glucose was quantified at 0 (before and after administration of glucose), 30, 60 and 90 min. Glucometer was used to record the assessments. In this study, the following groups were formed: (a) Control group, which received only glucose; (b) Treatment 1 group, which was given either unpasteurized sweet xoconostle (white/red) or pasteurized juice with a glucose load; and (c) Treatment 2 group, which was given either unpasteurized sweet xoconostle (white/red) or pasteurized juice 15 min prior to the administration of glucose. Pasteurized and unpasteurized juices were lyophilized before administration. Micropipette was used to administer a dose of 100 mg kg^{-1} of the juice. Every 15 min, blood glucose sample was collected through the caudal vein. Through the study, animals were fed *ad libitum*. Data analysis involved comparing the means of the groups without treatment to the groups treated with fresh and pasteurized juice. Specifically, analysis focused on comparing the means of the areas under the glucose gain curve for all groups. Area was computed using trapezoidal rule [16,20].

3. Results and Discussion

3.1 Chemical and physical parameters

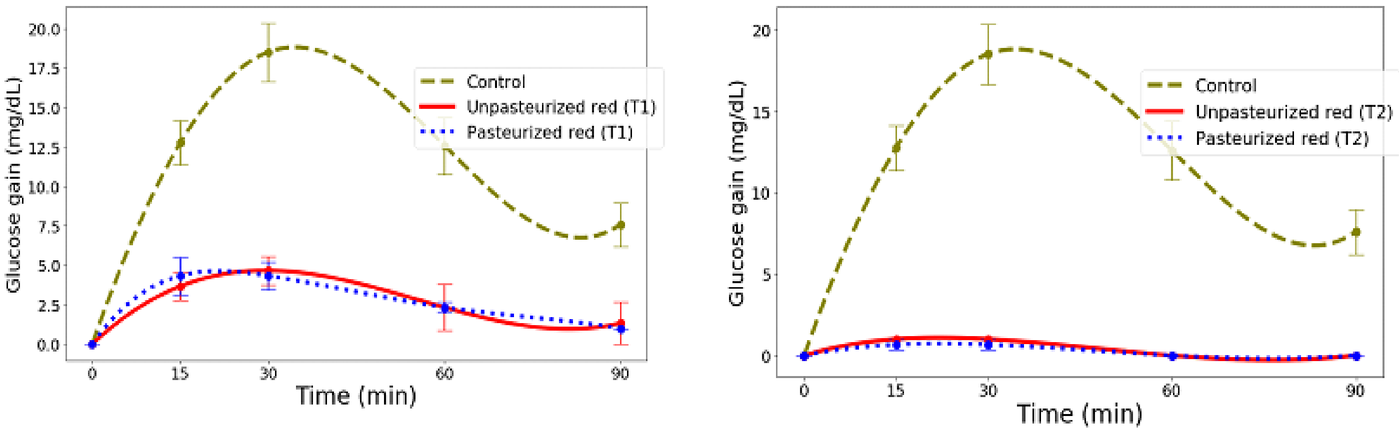
The analyzed fruits demonstrated significant heterogeneity for their average weights, which could be attributed to natural biological variability. A higher volume of juice was achieved from red tunillo, compared to white one. This allied with the higher weight of the red variant (Table 1). As expected, red tunillo contains betacyanins and betaxanthines, similar to that reported for Opuntia [21] (Table 1). This explains the similarity in the color of their pulps for the red variant. Microbial indicators led to negative results in the two cases, meaning that the process pasteurization was adequate [24]. However, a bacterial presence was detected in the unpasteurized juice, due to the presence of this type of bacteria in the environment. The pigment content in the analyzed red and white juices showed no changes after the pasteurization (Table 1) possibly because pH of the two juices was in the range of 3-7; in which, betalains were stable [25]. Additionally, pasteurization temperature was less than 85 °C; at which, only 10% degradation of this type of compounds were reported [26]. Stability of betalains in the pasteurization process is significant because these pigments are associated with antioxidant and antihyperglycemic characteristics [14,25]. It is possible to associate the content of betacyanins and betaxanthines to create a classification of the fruits. This compound (betacyanins and betaxanthines) is associated to antioxidant and antihyperglycemic characteristics, which can be used to differentiate and classify various types of these fruits [14]. It is noteworthy that the major betalains reported for the red variant include indicaxanthin, gomphrenin I and phyllocacthin [27].

Ratio of the total soluble solids to titrable acidity of the total soluble solids is an indirect form of measuring changes in organoleptic characteristics. Therefore, it was detected that the pasteurization process caused changes in the two variants of tunillo, indicating needs of sensory studies with trained panel to verify this effect (Table 1); similar to another study [21]. Hence, a rapid sensory evaluation by an untrained panel of 28 people did not report differences in flavor and color using blind assessment, which seemed to indicate that pasteurization did not produce organoleptic changes in the juice of both varieties; similar to what reported by Bobadilla using grapes juice [28]. They monitored degradation of flavonols, finding no changes before and after the pasteurization process. Although there are several reports that pasteurization process can cause organeptic and chemical changes [29-30], pasteurization process should be assessed in each biological matrix due to the difference in chemical and physical microclimates.

It is noteworthy that both variants of tunillo fruits exhibited considerably less acidity compared to Opuntia. The difference in acidity level could be attributed to the higher content of phenolic compounds present in Opuntia fruits. The study demonstrated that pasteurization did not cause changes in acidity level in Opuntia, possibly because Opuntia included a higher content of phenolic compounds, compared to tunillo [21]. The content of total phenolic compounds in Opuntia is higher than that reported for tunillo. This higher content might contribute to the stability of the redox ratio and antioxidant capacity in Opuntia fruits [14]. In this study, no significant changes were seen in the chemical and physical characteristics such as brix, pH and total flavonoids between the pasteurized and unpasteurized tunillo variants. However, it was detected that differences in the total phenolic compounds in red tunillo were due to pasteurization, in contrast to white tunillo. This suggested that the profile of phenolic compounds might be various in the two variants. The content of mallic acid changed in white tunillo but not in red tunillo (Table 1), possibly due to the degradation of this organic compound.

3.2 Anti-hyperglycemic effects

Regarding the anti-hyperglycemic effect, the glucose gain was lower in all groups, compared to the control group (Figure 1-3). In all cases, the area was less than control. Considering pasteurized and fresh juices, for each color and T1 or T2 treatments, there was no significant differences ($p \le 0.05$).



Figures 1. Anti-hyperglycemic effect of red tunillo administered at time zero (T1) and fifteen minutes before (T2) the glucose load.

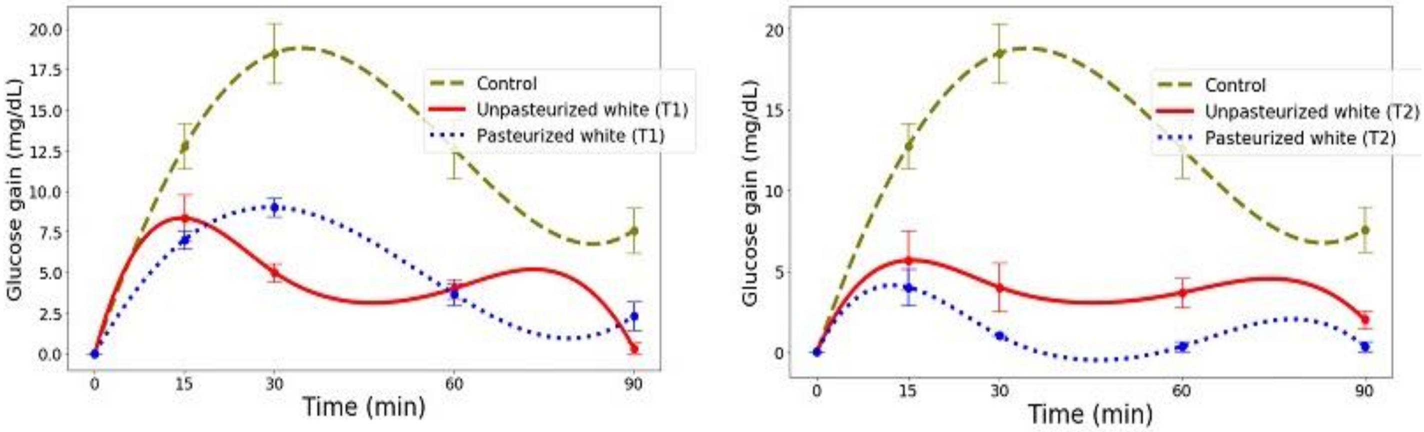


Figure 2. Anti-hyperglycemic effect of white tunillo administered at time zero (T1) and 15 fifteen before (T2) the glucose load.

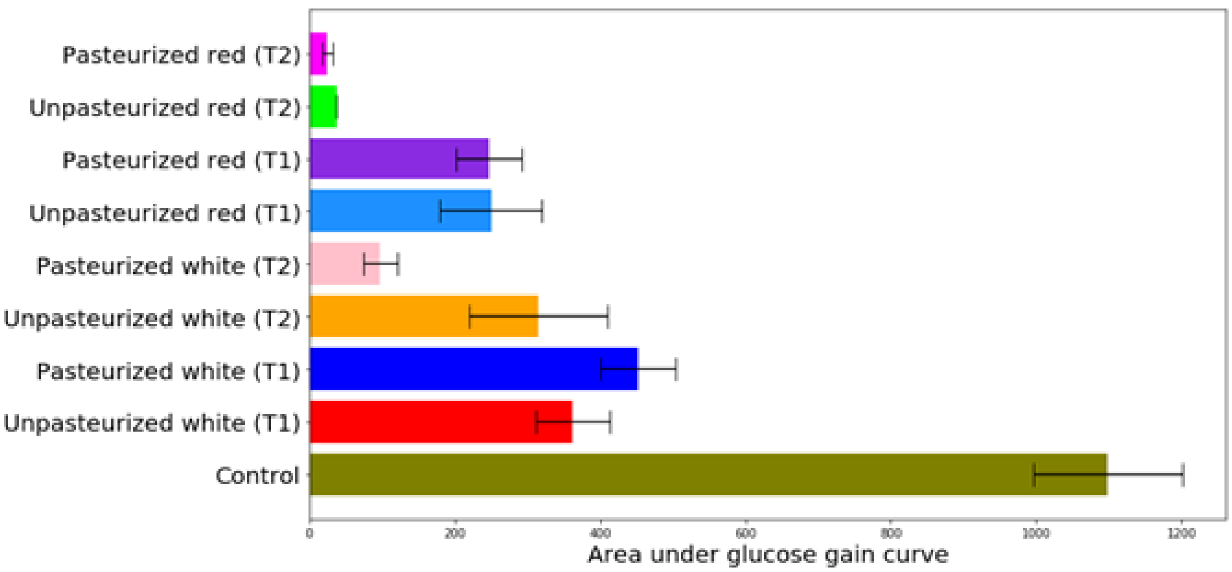


Figure 3. Area under glucose gain curve†

†A different one-tail test hypothesis was performed, where the null hypothesis states that there is no difference between the area associated with the red juice (pasteurized or unpasteurized) following T2 and the rest of the cases, and the alternative hypothesis states that this area is smaller in the case of the red juice following T2. With a significance-level of 5%, the null hypothesis is rejected in favor of the alternative hypothesis in all the cases.

Table 1. Physical and chemical parameters of pasteurized and unpasteurized juice of sweet xoconostle or tunillo (*S. stellatus*) white and red varieties

	White- tunillo		Red- tunillo	
Physical				
Fruit weight(g)	106.05 ± 10.4		111.1 ± 11.33	
Pulp weight (g)	80.31 ± 8.19		81.3 ± 8.08	
Volume juice (ml)	530 ± 137		760 ± 97	
Chemical	Pasteurized	Unpasteurized (Fresh)	Pasteurized	Unpasteurized (Fresh)
TSS† (°Brix)	9.64 ± 1.43	10.89 ± 2.25	9.5 ± 0.30	9.22 ± 0.04
TA‡ (% mallic acid)	0.05 ± 0.01	0.08 ± 0.01*	0.047 ± 0.00	0.047 ± 0.00
TSS/TA	193	136	202	196
pH	4.02 ± 0.13	4.22 ± 0.22	4.24 ± 0.05	4.21 ± 0.30
Total phenolics content (mgEAG.ml^{-1})	1.19 ± 0.46	1.35 ± 0.45	3.15 ± 0.02*	4.86 ± 0.01
Total flavonoids content (mgEQ.ml^{-1})	0.015 ± 0.01	0.013 ± 0.01	0.62 ± 0.01	0.64 ± 0.03
Betacyanins (μg.g^{-1})	0.002 ± 0.00	0.002 ± 0.000	0.20 ± 0.00	0.21 ± 0.00
Betaxanthins (μg.g^{-1})	0.001 ± 6×10^{-5}	0.001 ± 7×10^{-3}	0.188 ± 6×10^{-5}	0.192 ± 0.001

† total soluble solids
‡ titrable acidity
* Indicates differences between pasteurized and fresh in the same variety, student's test α = 0.05

In each group, pasteurized and unpasteurized tunillos showed similar behaviors. For red and white variants of tunillos, Treatment 2 carried out better than Treatment 1 due to the absorption time of active ingredient(s) and of the glucose in the extract. Treatment 2, which involved administeration of the juice 15 min before glucose doeses with better results (Figure 3). This suggested that consuming the juice before eating might lead to a better glycemic control in drug treatments. The best results were achieved for the red tunillo juice. A similar effect was reported in lyophilized pulps from Opuntia [21]. Regarding this effect, the current research group detected similar results in tunillo seeds [14] and lyophilized pulps, where it was observed that the characteristics of red tunillo in relation to its antihype-rglycemic characteristics were better than those of the other tunillo variants, meaning that the role of betaxanthin and betacyanin were possibly important. The current study also reported correlations between the antioxidant capacity and this effect [14]. Decreases of glucose levels were seen by the present investigators in other Cactaceae species such as *Opuntia* spp. [21], suggesting that associated taxa produced chemically and pharmacologically active compounds. In general, this effect is frequently associated with the antioxidant capacity derived from the content of phenolic compounds and organic acids [16].

4. Conclusion

In conclusion, results of the study have suggested that red and white tunillo juices can help regulate blood sugar levels. Pasteurization process does not seem to affect chemical and pharmacological characteristics of the juice, making it a viable option for the preservation and potential uses in production of functional drinks from *S. stellatus* fruits. However, it is necessary to study the juice shelf-life to assess its effects on animal diabetic models.

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6. Authors Contributions

All authors reviewed the results and approved the final version of the manuscript. Study conception and design, JAME and FDLS; data collection, SIGC, EÁR, FRC and RCA; analysis and interpretation of results, SIGC and SIGC; draft manuscript preparation, SIGC, JAME and FDLS.

7. Conflict of Interest

The authors report no conflicts of interest.

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اثر پاستوریزاسیون بر خواص ضدهیپرگلیسمی و شیمیایی آب میوه خوکونستل (*استنوسریوس استلاتوس*)

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چکیده

سابقه و هدف: اثر ضد هیپرگلیسمی به میوه پری‌هیسپانیک[۱] خوکونوستل یا تونیلو (استنوسریوس استلاتوس، فیفر، ریکوبونو) منسوب است. این میوه دارای انواع مختلفی است که از نظر رنگ متمایز هستند. میوه خوکونوستل بسیار فاسد شدنی هستند. لذا هدف از این مطالعه بررسی اثرات کاهنده قند خون آب خوکونوستل قبل (تازه) و بعد از پاستوریزاسیون بود. این مطالعه بر روی انواع سفید و قرمز خوکونوستل متمرکز بود.

مواد و روش ها: در این مطالعه، روش شامل جمع آوری آب میوه از میوه خوکونوستل و به دنبال آن پاستوریزاسیون بود. پارامترهای شیمیایی، فیزیکی و میکروبی برای آب میوه و توانایی کاهش سطح گلوکز مویرگی (اثر ضد هیپرگلیسمی) در موش‌های صحرایی نر ویستار بررسی شد.

یافته‌ها و نتیجه‌گیری: فرآیند پاستوریزاسیون منجر به کاهش محتوای فنلی کل در رقم قرمز میوه خوکونوستل شد، در حالی که رقم سفید افزایش محتوای اسید مالیک را نشان داد. علیرغم این تغییرات، آب میوه‌های تازه و پاستوریزه هد دو رقم در مقایسه با گروه شاهد، سطح گلوکز خون پایین‌تری را نشان دادند. رقم قرمز اثر ضد قند خون قوی‌تری را نشان داد. در نتیجه، پاستوریزاسیون بر اثرات دارویی آب خوکونوستل تأثیری نداشت و آن را به یک روش نگهداری مناسب بدون به خطر انداختن ویژگی های ضد هیپرگلیسمی تبدیل کرد. نتیجه این تحقیق یک روش نگهداری را پیشنهاد می‌کند که ضمن حفاظت از اثرات ضد قند خون آب میوه، در عین حال عمر مفید آن را نیز افزایش می‌دهد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- سطح گلوکز خون
- پاستوریزاسیون
- میوه پیش‌هیسپانیک
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