

Article

Extracts of *Hechtia* spp. as Novel Coagulants Reduce the Pollutant Load of Whey

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Abstract

Traditional coagulant calf rennet, which is used in cheese production, is currently facing the problem of an unsustainable source. In addition, the production of cheese with calf rennet produces whey with high biochemical (BOD) and chemical oxygen demand (COD) values. For these reasons, plant extracts have been investigated as sustainable sources of coagulants for milk. However, there are few reports on the changes in the COD and BOD of whey when plant extracts are used. For this reason, this study investigated the potential of extracts from two *Hechtia* species native to Mexico (*H. glomerata* and *H. podantha*) as sustainable milk coagulants for cheese production, with the aim of simultaneously reducing the pollutant load of residual whey. The milk coagulation efficiency of the extracts of the two *Hechtia* species was investigated, and in addition, their effects on cheese texture and color, and the composition of the residual whey, including BOD and COD, were evaluated. Most extracts of *H. podantha* showed adequate milk coagulation and yielded fresh cheese with textural properties comparable to those of cheese produced with conventional calf rennet. A significant reduction in carbohydrate content was achieved when *H. podantha* extracts were used. As a result, a significant decrease in the BOD and COD values was achieved. In some cases, a reduction of up to 1.78 times compared with those of the control was achieved. The results of this study show that *H. podantha* is a promising source of natural coagulants for sustainable cheese production, offering a dual benefit by providing an alternative to conventional rennet and reducing the environmental impact of whey.

Keywords: COD; BOD; *Hechtia glomerata*; *Hechtia podantha*; coagulation



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1. Introduction

In the dairy industry, cheese production is the most important area, but in recent years, controversies surrounding rennet production have increased, in addition to increased costs and a limited supply [1]. For these reasons, several plants have been evaluated as sources of coagulants for use in cheese production, such as the flowers of *Cyanara cardunculos* L. used by Alvarenga et al. [2] for the production of Serpa cheese, extracts of *Cynara humilis* for the production of sheep cheese [3], and *Nigella sativa* L. for the production of sheep curd [4]. Recently, there has been an intensified effort to find new milk coagulants, which is why various fruit and vegetable extracts, such as *Cynara cardunculus* var. scolymus, *Ananas*

comosus (L.) Merr., *Carica papaya* L., and *Ficus carica* L., and coagulants for the production of vegan products, such as *Pleurotus ostreatus* Jacq. ex Fr., have been evaluated [5].

The coagulant is decisive for the final quality of the cheese, as this step separates the milk into curd and whey [6]. Considering that extracts from *Cyanara cardunculos* have greater proteolytic activity than animal rennet does, these extracts are the most studied [7]; for example, this is the reason why these extracts produce a cheese with high moisture and a soft texture, similar to what Borges et al. [8] reported. Ordiales et al. [9] reported that when an aqueous extract of *Cyanara cardunculos* was used in the production of “Torta del Casar”, high creaminess was obtained. Tejada et al. [10] noted that aqueous extracts and powders of *Cyanara cardunculos* yield a sheep’s milk cheese (lot P) that is creamier than cheese made with animal rennet. On the other hand, it has also been reported that during the ripening of Los Pedroches cheese, there is no difference in proteolysis between cheeses made with animal or vegetable coagulant [11].

It has been reported that the texture of sheep’s milk cheese made with the same vegetable coagulant is not constant. For this reason, Barracosa et al. [12] proposed an experimental design to predict the texture of Protected Designation of Origin cheeses from the Mediterranean region. In addition, other cheeses produced with vegetable coagulant exhibit altered textural properties, as reported, for example, by Gutierrez-Méndez et al. [13], who revealed that vegetable coagulants do not affect the yield of cheese, but the viscoelastic properties of the cheese are altered. In terms of texture, García et al. [14] noted that fresh goat’s milk cheese made with vegetable coagulant has greater chewiness, gumminess, and hardness than cheese made with animal or microbial rennet. Importantly, a bitter taste is perceived when a vegetable coagulant is used for the coagulation of cow’s milk, and Colombo et al. [15] reported changes in the springiness and microstructure of miniature cheeses.

In Mexico, several authors have reported that plants from the Bromeliaceae family, such as the “guapilla” (*Hechtia podantha* Mez.), are used as coagulants for the production of cheese [16,17]. However, nothing has been reported on the coagulation process or their enzymatic effect on milk. Considering the large number of sources of milk coagulants extracted from vegetables and the variety of textural properties of cheese made with plant extracts, evaluating the physicochemical characteristics of cheeses obtained with plant coagulants is important. However, the production of cheese generates whey, which contains milk proteins, carbohydrates, mineral salts, and some water-soluble vitamins. For this reason, waste whey is an agent with high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which poses a risk to the environment if discharged directly into the sewer system or a body of water [18]. Therefore, the aim of this study was to evaluate the effects of aqueous extracts of two Bromeliaceae, *Hechtia glomerata* Zucc. and *Hechtia podantha* Mez., on the structural properties of fresh cheese made from cow’s milk and the residual macromolecules in the waste whey, with the aim of reducing the BOD and COD of the residual whey, as an alternative approach to sustainable cheese production.

2. Materials and Methods

Wild *Hechtia glomerata* Zucc. and *Hechtia podantha* Mez plants were used for this study. The *Hechtia glomerata* Zucc. plants were collected in the municipality of Cardonal in the Mexican state of Hidalgo at 20°33′11.7″ N and 99°14′1.878″ E and 2100 m above sea level. The *Hechtia podantha* Mez plants were collected at 20°22′38.201″ N and 99°14′10.878″ and 1840 m above sea level in the municipality of Chilcuautila in the state of Hidalgo, Mexico. Both plants were identified in the QMEX Herbarium “Dr. Jerzy Rzedowski” at the Universidad Autónoma de Querétaro (UAQ, Mexico). The voucher numbers of the repositories for *H. glomerata* and *H. podantha* are QMEX00007826 and QMEX00007814, respectively.

2.1. Obtaining the Extracts

Seven extracts were obtained from each *Hechtia* plant. First, the clean and thornless leaves of the plants were blended in a commercial blender (model Oster BLSTBPST-013, 127 V power), and the liquefied sample was then filtered through a cotton mesh and labeled JVL. For the second extract, a sample was pressed and then filtered through a cotton mesh and labeled JVP. For the third sample, only the parenchymatous tissue was mixed and filtered through a cotton mesh (referred to as EP), whereas for the fourth extract, only the parenchymatous tissue was cut into cubes of approximately 0.5 cm (referred to as PT). In addition, each whole plant was cut into cubes of approximately 0.5 cm, and the cubes were used for the coagulation process (referred to as HT). The chlorenchymatous tissue was separated from the parenchymatous tissue and then cut into cubes of approximately 0.5 cm (referred to as ET). Finally, the rosette base was mixed, and the liquefied sample was then filtered through cotton mesh. This sample was designated RB. All extracts were obtained by pressing after mixing, or the cubes were incorporated directly into the milk, and no solvent was used. The pH of the pressed samples was adjusted to 5.5 with phosphate buffer, and the samples were stored under refrigeration (4 °C) until use.

The parts considered parenchymatous and chlorenchymatous tissue are shown in Figure 1.

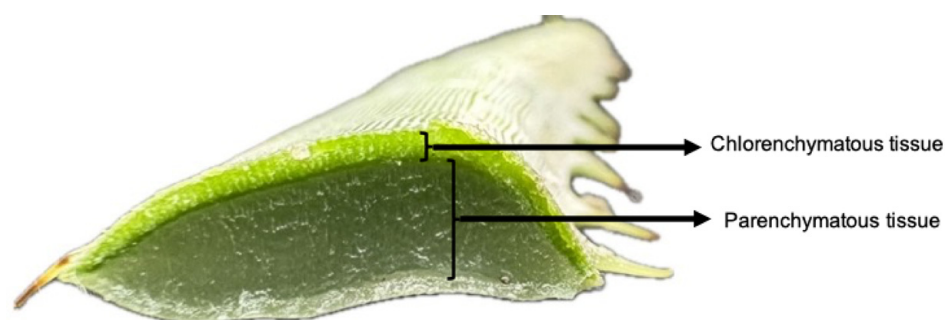


Figure 1. Parts of the *Hechtia* leaf.

2.2. Cheese Production Process

The method described by Martínez-Ruiz and López-Díaz [19] was used to prepare the cheese, with slight modifications. To 250 mL of pasteurized cow's milk, 0.2% CaCl_2 and 0.1% KNO_3 were added. The milk was then brought to a constant temperature (38 °C), and the plant extracts were added. The milk with the plant extracts was incubated until the coagulation process was complete. A control cheese was made with calf rennet under the same conditions. A flowchart of the cheese production process is shown in Figure 2.

The yield was calculated using Equation (1).

$$\%Yield = \frac{m_2 * 100}{m_1} \quad (1)$$

where m_1 = milk weight and m_2 = curd weight.

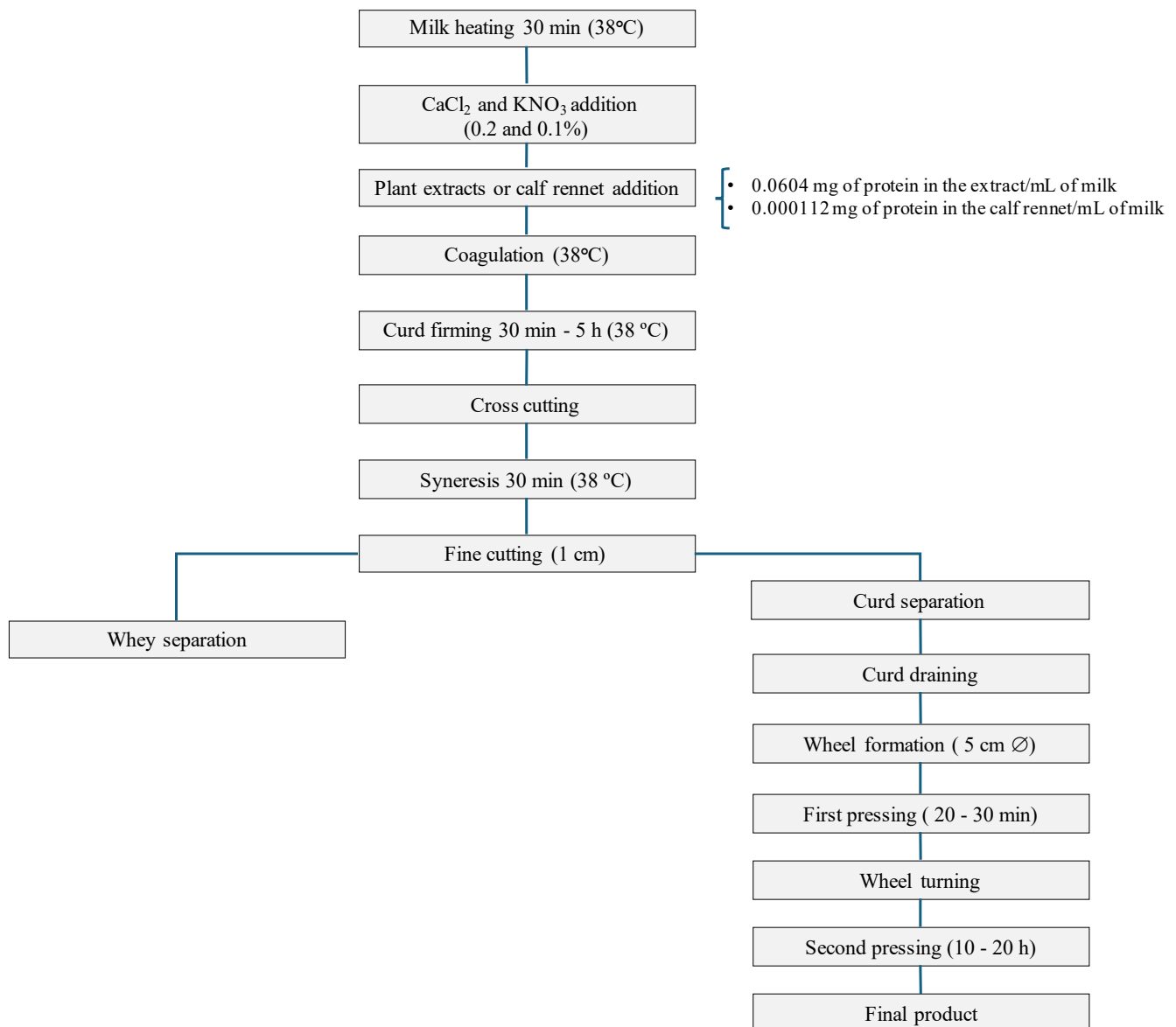


Figure 2. Flowchart of the cheese production process.

2.3. Texture Analyses

The textural properties of the cheese produced, such as hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness (TPA), were determined with a CT3 Texture Analyzer (Brookfield Ametek, Middleborough, MA, USA). Two consecutive compression tests were performed with probe number TA4/1000-cylinder 38.1 D, 20 mm L. The velocity reached 2 mm/s, and the compression index was 50%. The TPA parameters were calculated using the device's texture analysis software (TexturePro CT Software V1.9 Build 35).

2.4. Determination of Color in Cheeses

A 5.7 cm cylindrical cheese was used to determine its color using a Konica Minolta colorimeter (model: CR-410 series, Minolta Corp., Ltd., 2002, Tokyo, Japan). The color values L^* , a^* , and b^* of all the samples were measured directly with this device. The color differences between the cheese made with the *Hechtia* extracts and the control cheese

(made with calf rennet) were calculated using the CIE Delta E (CIE ΔE) according to Equation (2) [20].

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

where

$$\Delta L^* = L^*_{m1} - L^*_{m2};$$

$$\Delta a^* = a^*_{m1} - a^*_{m2};$$

$$\Delta b^* = b^*_{m1} - b^*_{m2}.$$

The C^* and h°_{ab} values were calculated according to the equations described by Kim et al. [20]; for the C^* value, Equation (3) was used:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

For the h°_{ab} values, Equation (4) was used.

$$h^{\circ}_{ab} = \tan^{-1} \frac{b^*}{a^*} \quad (4)$$

2.5. Determination of the Remaining Macromolecules in Whey

The concentration of the remaining macromolecules in the whey was determined according to the methods described by the following authors. For protein, the method described by Bradford [21] was used, i.e., 3000 μL of Bradford reagent was added to 100 μL of whey, the mixture was allowed to rest for 10 min, and then the absorbance was recorded at 595 nm. A mixture of 100 μL distilled water and 3000 μL was used as a blank. Bovine serum albumin was used as an external reference sample. The phenol–sulfur method described by Dubois et al. [22] was used for carbohydrate determination. To 400 μL of whey, 400 μL of phenol (5%) and 2 mL of sulfuric acid were added. The mixture was kept at room temperature for 10 min, shaken vigorously, and then held at room temperature for 30 min. The absorbance was then measured at 490 nm. Lactose was used as an external reference sample.

2.6. Determination of the BOD and COD of the Whey

Method 5220D of the standard methods [23] was used for the assessment of BOD and COD. In brief, a dilution of the whey sample (1:1000) was performed. In a 10 mL tube, 2.5 mL of this dilution was mixed with 1.5 mL of the digester solution ($\text{K}_2\text{Cr}_2\text{O}_7$, H_2SO_4 , and HgSO_4) and 3.5 mL of the sulfur reagent (Ag_2SO_4 and H_2SO_4). The mixture was heated to 150 $^{\circ}\text{C}$ for 2 h, after which the samples were cooled to room temperature. The samples were then centrifuged at 6000 rpm for 10 min (Marca, Modelo). The supernatant was recovered, and the absorbance was measured at 600 nm in a spectrophotometer (UV/Vis spectrophotometer SP-3000 nano, OPTIMA Tokyo Japan). With the data obtained, the COD was determined using a pattern curve, and the BOD value was calculated theoretically by multiplying the COD value by the organic matter content (77.5%).

2.7. Statistical Analysis

A completely randomized experimental design with one factor was used for the experiment. The factor extract was assessed at seven levels (JVL, JVP, EP, PT, HT, ET, and BR). Unidirectional analysis of variance (ANOVA) followed by post hoc Tukey–Kramer analysis was performed, and $p < 0.05$ was used to designate statistical significance. All analyses were performed using Origin Pro software, v. 2021 (OriginLab Corporation, Northampton, MA, USA.). All the experiments were performed in triplicate.

3. Results and Discussion

3.1. Yields of Extracts

As shown in Table 1, the yield of the extracts of *H. podantha* was greater than that of *H. glomerata*. However, the EP in both plants presented the highest yield of extract per gram of sample; in most cases, the yield was twice as high as that from the other plant parts.

Table 1. Yields of extract from different plant parts of the two *Hechtia* species.

Species	Sample	Yield (mL of Extract/g of Sample)
<i>H. glomerata</i>	JVL	0.22
	JVP	0.22
	EP	0.62
	PT	WE
	HT	WE
	ET	WE
	BR	0.18
<i>H. podantha</i>	JVL	0.30
	JVP	0.30
	EP	0.66
	PT	WE
	HT	WE
	ET	WE
	BR	0.35

WE: without extract; as the plant sample was cut into cubes and added directly to the milk, no extract was obtained.

The higher yield from the EP is due to the presence of water reservoirs in this part of the plant, which increases the amount of extract. It has been reported that the yield of the extract depends on the solvent used [24] and the part of the plant studied [25]. The above results are consistent with the results of this study, as differences in yield were observed between plant species and between plant parts.

In addition to the yield of the extracts, the protein concentration in the extracts was also determined, with concentrations between 0.89 and 1.51 mg/mL being obtained. Considering that *Hechtia* species belong to the *Bromilaceae*, the protein concentration in the extracts was higher than that in extracts from another *Bromilaceae*, *Bromelia hieronymi*, with a concentration of 0.47 mg/mL reported by Bruno et al. [26]. However, it is similar to extracts obtained from *Moringa oleifera* and *Carica papaya*, whose concentrations were reported as 1.1 and 1.2 mg/mL, respectively [27], but were lower than those reported by Silva et al. [28] for extracts of *Cynara cardunculus* (9 mg/mL). The amount of protein recovered is important, as this may be an important factor in the recovery of the proteolytic enzyme.

3.2. Coagulation Time Test

As shown in Table 2, the clotting time was between 2 and 9 h. In some cases, such as the RB extract of *H. podantha*, the clotting time was reduced by up to 70% compared with that of the extracts of *H. glomerata*. Proteases are not present in all parts of the plant; therefore, most extracts showed no enzymatic activity on milk proteins when *H. glomerata* extracts were used, but when *H. podantha* extracts were used, 70% of the extracts showed procoagulant activity.

The amount of protein used for the coagulation process is crucial. For this reason, according to Hoxha et al. [29], the best coagulation activity for 10 mL of milk is achieved at a concentration of 29.6 µg of protein in an extract of *Cirsium* spp. However, higher concentrations (148 µg or 296 µg) of protein for the same amount of milk lead to greater instability of

the casein network due to its destruction, which is reflected in the paste firmness parameter. On the other hand, Nazish et al. [30] noted that the protease concentration influences not only the viscosity of the curd but also the yield, syneresis, and coagulation time.

Table 2. Time required for the coagulation of the proteins in milk with several extracts of two species of the genus *Hechtia*.

Species	Extract	Coagulation Time (h)
<i>H. glomerata</i>	JVL	WC
	JVP	9
	EP	WC
	PT	WC
	HT	WC
	ET	WC
	RB	WC
<i>H. podantha</i>	JVL	3
	JVP	8
	EP	5
	PT	9
	HT	WC
	ET	WC
	RB	2

RB: rosette base; WC: without clotting.

Several reports indicate different coagulation times when plant extracts are used. For example, El-Kholy [31] reported that the coagulation time of cow's milk when an extract from artichoke styles is used is between 40 and 50 min, similar to an extract from *Solanum incacum* with a coagulation time of 40 min [32]. On the other hand, studies of the leaf extract of *Calotropis gigantea* have shown that the coagulation time of suspensi soft cheese is between 14 and 16 min [33]. However, it was also reported that a partially purified enzyme from sunflower had a clotting time of 3.60 h [34], whereas the extract of *Cirsium* spp. had a clotting time of 6 h [29]. In another study in which an extract of *Whitania coagulans* was used to coagulate camel and bovine milk, the clotting time of the cheese was 4 h [35]. In view of this, extracts of *Hechtia* could be an option for the production of fresh cheese with a reasonable coagulation time.

These results indicate that RB has a relatively high concentration of enzymes responsible for coagulation, which is why the coagulation time is significantly reduced when this part of the plant is used. This is an important finding, as this study proves that this part of the plant is useful for efficiently obtaining the enzyme responsible for the hydrolysis of casein.

3.3. Color of the Cheese Manufactured

Table 3 summarizes the color parameters of cheeses made with extracts from the two *Hechtia* species: calf rennet and a commercial cheese. The cheeses had a whitish-yellowish color. The color analysis of the cheeses revealed that the luminosity of the cheeses produced with *H. podantha* and *H. glomerata* was, in most cases, significantly lower than that of the commercial cheese. However, a smaller difference was observed between the cheeses made with *H. podantha* and *H. glomerata* and those made with calf rennet. The lower b* values of the cheeses produced with *H. podantha* and *H. glomerata* extracts indicate that their coloration is less yellowish than that of the commercial cheese and the cheeses produced with calf rennet. Only two extracts of *Hechtia podantha* produced a slight green coloration of the cheese (JVL and PT), as indicated by the negative value of a*.

Table 3. L* a* b* coordinates and other color parameters of cheese produced with different coagulants and commercial cheese.

Species	Extract	L*	a*	b*	ΔE	C*	h*
<i>H. glomerata</i>	JVP	21.80 $\pm$ 0.78 ^f	0.36 $\pm$ 0.53 ^f	18.82 $\pm$ 0.68 ^f	42.11 $\pm$ 0.88 ^a	18.83 $\pm$ 0.67 ^f	1.55 $\pm$ 0.03 ^a
	JVL	26.97 $\pm$ 0.85 ^e	−2.68 $\pm$ 0.46 ^g	25.22 $\pm$ 0.98 ^e	35.07 $\pm$ 0.88 ^c	25.37 $\pm$ 0.95 ^e	−1.46 $\pm$ 0.02 ^d
<i>H. podantha</i>	JVP	26.05 $\pm$ 0.66 ^e	2.87 $\pm$ 0.44 ^e	19.68 $\pm$ 0.74 ^f	38.40 $\pm$ 0.14 ^b	19.89 $\pm$ 0.78 ^f	1.43 $\pm$ 0.02 ^b
	EP	41.62 $\pm$ 0.99 ^d	6.70 $\pm$ 0.20 ^c	41.05 $\pm$ 0.40 ^c	11.76 $\pm$ 0.69 ^g	41.60 $\pm$ 0.37 ^c	1.41 $\pm$ 0.01 ^b
	PT	71.59 $\pm$ 0.72 ^a	−0.70 $\pm$ 0.12 ^f	31.30 $\pm$ 0.99 ^d	32.22 $\pm$ 0.12 ^d	31.30 $\pm$ 0.99 ^d	−1.55 $\pm$ 0.03 ^e
	RB	21.76 $\pm$ 0.35 ^f	14.84 $\pm$ 0.68 ^a	18.90 $\pm$ 0.86 ^f	26.79 $\pm$ 1.12 ^e	24.05 $\pm$ 0.32 ^e	0.90 $\pm$ 0.04 ^c
Control (calf rennet)		47.91 $\pm$ 0.26 ^c	9.60 $\pm$ 0.47 ^b	50.51 $\pm$ 0.96 ^b	---	51.42 $\pm$ 0.98 ^b	1.38 $\pm$ 0.01 ^b
Commercial cheese		65.25 $\pm$ 0.79 ^b	4.06 $\pm$ 0.80 ^d	54.34 $\pm$ 0.58 ^a	18.64 $\pm$ 0.58 ^f	54.50 $\pm$ 0.58 ^a	1.50 $\pm$ 0.01 ^a

Values in the same column followed by different letters differ significantly ($p < 0.05$) according to Tukey's method.

The color of cheese coagulated with plant extracts depends on the type of plant, the concentration of the extract, and the ripening process. However, according to some authors, the lightness value (L*) decreases when the amount of added plant extract increases [36]. With respect to the a* value, most of the cheeses produced in this study had a positive value, indicating that they had a predominantly yellow, although not intense, color. This is in contrast to the reports of Correia et al. [37], who found that cheeses made with thistle flower extracts had a predominantly green color, with negative values for a*. On the other hand, the positive values for b* agree with those of Guiana et al. [38], who reported that these positive values indicate a yellow coloration. On the basis of the results obtained, it can be concluded that the color parameters meet the requirements of the industry, as dyes can be used to standardize the color of the cheese.

3.4. Textural Profile Analysis (TPA)

One of the most important characteristics for consumer acceptance of cheese is the textural properties that influence the composition of the waste whey. Table 4 shows the TPA values, which indicate that the cheeses produced with the extracts of the *Hechtia* species did not have the same hardness after the first cycle. In most cases, the degree of hardness loss was greater than that in the control cheese. In most cases, however, they were more adhesive than the control and commercial cheeses. No significant differences were found between the cheeses made with the extracts and the control cheese in terms of cohesiveness, springiness, gumminess, or chewiness.

Table 4. Texture profile analysis values of curd prepared with different types of coagulants.

Species	Extract	Hardness (N)		Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
		Cycle 1	Cycle 2					
<i>H. glomerata</i>	JVP	11.33 $\pm$ 0.34 ^c	8.20 $\pm$ 0.96 ^b	1.97 $\pm$ 0.78 ^b	2.10 $\pm$ 0.50 ^b	−0.56 $\pm$ 0.02 ^a	23.65 $\pm$ 5.11 ^b	−13.17 $\pm$ 2.40 ^{ab}
	JVL	11.91 $\pm$ 0.25 ^{bc}	7.65 $\pm$ 1.08 ^b	8.20 $\pm$ 3.24 ^a	1.87 $\pm$ 0.25 ^b	−0.58 $\pm$ 0.52 ^a	22.24 $\pm$ 2.67 ^b	−12.03 $\pm$ 10.80 ^{ab}
<i>H. podantha</i>	PT	17.26 $\pm$ 0.38 ^a	15.44 $\pm$ 1.91 ^a	0.60 $\pm$ 0.20 ^b	2.47 $\pm$ 0.96 ^b	−0.47 $\pm$ 0.20 ^a	42.80 $\pm$ 17.44 ^{ab}	−17.97 $\pm$ 1.16 ^{ab}
	JVL	11.58 $\pm$ 0.46 ^c	7.73 $\pm$ 1.38 ^b	4.47 $\pm$ 3.85 ^{ab}	1.66 $\pm$ 0.36 ^b	−0.42 $\pm$ 0.36 ^a	19.19 $\pm$ 4.05 ^b	−8.13 $\pm$ 7.38 ^a
	EP	10.78 $\pm$ 0.47 ^c	7.10 $\pm$ 0.06 ^b	3.10 $\pm$ 2.34 ^{ab}	2.43 $\pm$ 0.27 ^b	−0.75 $\pm$ 0.13 ^a	22.46 $\pm$ 5.83 ^b	−16.90 $\pm$ 5.49 ^{ab}
Control (calf rennet)		12.82 $\pm$ 0.64 ^b	9.22 $\pm$ 3.61 ^b	1.83 $\pm$ 0.68 ^b	2.08 $\pm$ 1.26 ^b	−0.59 $\pm$ 0.26 ^a	17.16 $\pm$ 1.60 ^b	−13.43 $\pm$ 3.20 ^{ab}
Commercial cheese		9.24 $\pm$ 0.35 ^d	8.25 $\pm$ 0.58 ^b	0.90 $\pm$ 0.56 ^b	6.26 $\pm$ 2.21 ^a	−0.13 $\pm$ 0.59 ^a	57.38 $\pm$ 18.70 ^a	−23.87 $\pm$ 1.00 ^b

Values in the same column followed by different letters differ significantly ($p < 0.05$) according to Tukey's method.

It has been reported that the creaminess of cheese made with plant extracts is rated higher than that of cheese made with animal rennet [39–41]. In this study, similar results were obtained for cheeses made with extracts of *Hechtia* species. In addition, Galán et al. [42] reported that cheese made with plant coagulants is softer and creamier than cheese made with animal coagulants. These findings are consistent with the results of this study, as the cheese made with *Hechtia* extracts was creamier than the commercial cheese. According to Mazorra-Manzano et al. [43], the softness of cheese made with extracts of *Hechtia* species is because fresh curd coagulated with vegetable coagulants generally is quite soft and then hardens when stored for at least three days.

Considering the texture and color characteristics of the cheese produced in this work and considering that the value of the market for cream cheese was estimated at USD 7.3 billion in 2024 [44], the acceptability of the product could be high in terms of the texture obtained, and the color could be masked with natural colorants.

For the standardization of fresh cheese production, the purification of the proteolytic enzyme responsible for the hydrolysis of casein must be carried out in further studies using several columns that ensure adequate control of the process and allow the identification of its chemical properties.

3.5. Residual Macromolecules in the Whey

As shown in Table 5, the carbohydrate content in the whey decreased significantly when most extracts of *H. podantha* and *H. glomerata* were used as coagulants for the production of fresh cheese. Only when the extract obtained from the rosette base (RB) was used as a coagulant was the carbohydrate content in the whey higher than that in the whey obtained from the control cheese. A slight decrease in the residual protein content in the whey was observed in most of the cheeses made with the extract of *H. podantha*. Compared with that of the control cheese, the only usable extract for the production of cheese from *H. glomerata* presented a significantly decreased residual protein content in the whey.

Table 5. Remaining protein and carbohydrates in the whey.

Species	Extract	Residual Protein g of Protein/L of Whey	Residual Carbohydrates g Eq Lactose/L of Whey
<i>H. glomerata</i>	JVP	0.60 ± 0.02 ^c	39.39 ± 0.47 ^f
	JVL	0.74 ± 0.01 ^b	75.19 ± 0.38 ^d
<i>H. podantha</i>	JVP	0.73 ± 0.02 ^b	98.61 ± 0.14 ^c
	EP	0.76 ± 0.01 ^b	56.49 ± 0.54 ^e
	PT	0.79 ± 0.04 ^b	56.32 ± 0.07 ^e
	RB	0.92 ± 0.02 ^a	149.49 ± 0.83 ^a
	Control (calf rennet)	0.78 ± 0.02 ^b	111.60 ± 0.69 ^b

Values in the same column followed by different letters differ significantly ($p < 0.05$) according to Tukey's method.

The remaining macromolecules in the whey are the result of curd formation during cheese production. However, their concentration depends on the quality and composition of the milk, the type of cheese produced, the molecular variants of casein, and the processing parameters [45]. For these reasons, the production of a control cheese with calf rennet is important. The concentration of macromolecules in whey is important, considering that the composition of wastewater is crucial for the formation of contaminated water, with the high biochemical oxygen demand of whey being a major problem due to its high degree of disposal and contamination [18]. Buchanan et al. [46] reported that the protein concentration in whey obtained during cheese production (6 and 10 g/L) is almost four times greater than that reported in this study, which is related to the cheese yield.

However, the lactose concentration in the whey from this study was higher than the values reported by the same authors for lactose in whey, which were between 44 and 46 g/L. However, the protein concentration in the whey obtained by coagulating cow's milk with extracts of *Hechtia* species was lower. The concentration of carbohydrates was high, which is not good considering that lactose is responsible for approximately 90% of the BOD and COD values.

3.6. BOD and COD Determination

The cheeses produced with the extracts of both *H. glomerata* and *H. podantha* presented a reduction in BOD and COD between 23 and 178% for most of the residual whey compared with the residual whey of the control (Table 6). Only the RB extract produced residual whey with a BOD and COD that was 12% higher than that of the control residual whey.

Table 6. Chemical and biochemical oxygen demand of whey.

Species	Extract	COD mg of O ₂ /L	BOD mg of O ₂ /L
<i>H. glomerata</i>	JVP	213,936.97 ± 37,676.15 ^{ab}	165,801.15 ± 29,199.02 ^{ab}
<i>H. podantha</i>	JVL	336,144.83 ± 28,916.07 ^{ab}	260,512.25 ± 22,409.96 ^{ab}
	JVP	235,010.61 ± 180,070.23 ^{ab}	182,133.22 ± 139,554.43 ^{ab}
	EP	150,427.37 ± 14,240.24 ^b	116,581.21 ± 11,036.19 ^b
	PT	339,993.90 ± 84,015.30 ^{ab}	263,495.27 ± 65,111.86 ^{ab}
	RB	473,748.97 ± 179,175.50 ^a	367,155.45 ± 138,861.01 ^a
Control (calf rennet)		418,899.77 ± 71,900.06 ^{ab}	324,647.32 ± 55,722.55 ^{ab}

Values in the same column followed by different letters differ significantly ($p < 0.05$) according to Tukey's method.

According to the report by Hoover et al. [47], the COD of the whey waste stream is high due to the high organic carbon content, reaching values of 122,000 mg/L, whereas Kazimierowicz et al. [48] reported that the COD of acid whey is between 50,000 and 100,000 mg/L. These values are lower than those determined in this study. However, the COD is determined by the type of whey (sweet or acid whey) produced during cheese processing and the origin of the milk. It also depends on the feeding of the animals, the season, and the stage of lactation [49].

The observed reduction in BOD and COD in the residual whey of the cheeses produced with extracts of *Hechtia* species compared with the cheeses produced with calf rennet is due to the lower concentration of carbohydrates in the residual whey of the cheeses produced with plant extracts. The differences in the BOD and COD values between the individual plant parts are related to the concentration/type of coagulating enzyme, the various organic accompanying substances, and the plant species. Since *Hechtia* species grow in open fields in semidesert areas and are dependent only on rainwater, the extraction of plant coagulant requires less land/water than does calf rennet production. Land is needed for the growth of the calf and its mother and for the production of grass as feed for the cow, and water is needed for irrigation of the grass, consumption by the cow, and cleaning of the calf and cow areas.

The benefit to the environment is greater. The regulatory hurdles for the use of *Hechtia* extracts depend on the country in which they are used. As regulatory control varies from country to country, in most cases, the declaration on the label is sufficient, but it must be properly monitored [50].

4. Conclusions

Both plants, *H. glomerata* and *H. podantha*, have high potential for use as milk coagulants in the production of fresh cheese. According to the TPA results, both plants produced cheese with properties most similar to those obtained using calf rennet (control). Although the protein concentration in the whey obtained from the curdled milk is similar when plant extracts and calf rennet are used, the lactose concentration in the waste whey is lower when one of the two *Hechtia* extracts is used than when calf rennet is used. For these reasons, using *H. glomerata* or *H. podantha* instead of calf rennet could improve the sustainability of the production of fresh cheese, considering that BOD and COD were lower in the cheese produced with *H. glomerata* and *H. podantha* than in the cheese produced with calf rennet.

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Abbreviations

The following abbreviations are used in this manuscript:

JVL	Juice Green Liquid (leaves blended and filtered)
JVP	Juice Green Press (leaves pressed and filtered)
EP	Parenchymatous Tissue (mixed and filtered)
PT	Parenchymatous Tissue (cut into cubes)
HT	Whole plant (cut into cubes)
ET	Chlorenchymatous tissue (cut into cubes)
RB	Rosette base (Rosette blended and filtered)

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