

# **Original Article**

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# Evaluation of the shelf-life of blackberry pulp fortified with physiologically active compounds

Evaluación de la vida útil de la pulpa de mora enriquecida con compuestos fisiológicamente activos

Restrepo, María Alejandra<sup>1</sup>, Luna-Ramírez Julio Cesar<sup>2</sup>, Quintero-Castaño Victor<sup>3\*</sup>.

<sup>1</sup>Química, gcbarong@gmail.com, ORCID 0000-0001-8987-5214, Universidad Industrial de Santander, Bucaramanga, Colombia. <sup>2</sup>Doctora en Química, amontano@uis.edu.co, ORCID 0000-0003-1694-7170, Universidad Industrial de Santander, Bucaramanga, Colombia.

<sup>3\*</sup>Doctora en Ciencias Física, claudia.gonzalez@upb.edu.co, ORCID 0000-0001-6099-907x, Universidad Pontifica Bolivariana, Bucaramanga, Colombia.

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	RESUMEN
Palabras clave:	La vida útil de un jugo de mora pasteurizado adicionado con calcio y vitamina C fue evaluado en
Pulpa,	el presente trabajo. El producto fue caracterizado fisicoquímica, microbiológica y sensorialmente
	a 25, 35 y 45 °C durante 30 días a 80% de humedad relativa. Se realizó un análisis de varianza
Mora,	para determinar la variable más afectada en el almacenamiento. Los resultados mostraron que
Vida util	los microorganismos mesófilos se detectaron el día 21 de almacenamiento a 15 °C, el día 18 a 25
	°C y el día 11 a 35 °C. Los modelos matemáticos integrados cinéticos y Arrhenius se obtuvieron
	para las variables fisicoquímicas L *, a *, b *, brix, pH y viscosidad. Los modelos cinéticos del
	comportamiento sensorial de la pulpa almacenada a 25 °C fueron obtenidos. Se pudo determinar
	sensorialmente que el producto fue rechazado en promedio para todos los descriptores de 12 días.

# Introduction

Processed products obtained from blackberry have been commercially exploited, to the point that most of the blackberry produced in Colombia is purchased by companies, like Postobon®, Compañía Nacional de Chocolates®, Casa Luker®, Agrofood®, Pascol®, and Alpina® [1]. It is clear that at present, according to reports from the Colombian Ministry of Agriculture in its platform "www.siembra.gov.co" no research, development and innovation work related to the agroindustrial exploitation of the blackberry is observed, especially in the Andean region, which has the highest production of this fruit [2].

Blackberry is a perishable product that can last in storage from 5 days in fresh state to 1 month in frozen state. This implies that there may be great loss due to the lack of existing technical capacity to preserve the organoleptic, microbiological, and nutritional characteristics of blackberry [3]. The Siembra platform by the Ministry of Agriculture in the area of harvest, post-harvest, and transformation management for the blackberry chain shows a demand in bio-prospection to develop agroindustry and transform this type of product. Development of new products with high added value for national and international markets is of interest for researches. Poor participation by national players is noted in the research on transformation alternatives for food, health, and industry compared to the participation by international players in this same chain. On this platform, it can be observed that contributions by national entities towards improving the agroindustrialization of the blackberry has been quite low. It can also be perceived that most efforts made in the chain have been in agricultural areas, like management of the production system, sanitary and phytosanitary management, and planting material and genetic improvement; the area of harvest management, postharvest and transformation would be fourth in importance according to the projects presented and the results published nationally [2].

Concern arises because blackberry producers and processors are not being supplied with knowledge so that they can obtain processed products with greater added value and with immersed scientific knowledge to obtain better economic benefit without involving intermediaries, who earn the greatest financial profit of selling the product to specialized markets. This implies that it is necessary for regional research, development, and innovation participants to become more involved in the generation of knowledge and interaction with the productive sector to secure better products, techniques, and production processes of transformation and generation of added value in the blackberry chain.

Blackberry has excellent nutritional characteristics, providing a substantial amount of water, carbohydrates, and fiber, but its most important contribution is in the content of anthocyanins, given that this group of molecules has been reported as antioxidants for humans, generating much interest from the scientific point of view.

One way to take advantage of these anthocyanins is to manufacture food products based on blackberries, high fruit content, and without the addition of water, caloric sweeteners and artificial preservatives to obtain foods that benefit the health of consumers; these foods are also called functional foods.

One of these products is fruit pulp, which is used as a base to prepare juices, nectars, compotes, ice cream, and pastries, among others. According to the Colombian technical standard 5468 "juices, nectars, purées (pulps), and fruit concentrates" to produce fruit pulp, it is necessary to extract the liquid and edible part of the fruit by mechanical means and without the addition of water; additionally, all the solids of the pulp must be contributed by the fruit. Furthermore, resolution 333 of 2011 by the Ministry of Social Protection "by which the technical regulation is established on the requirements of labeling or nutritional labeling that must be met for food packaged for human consumption" approves and regulates the addition of bioactive chemical compounds to fortify pulps, such as vitamins and minerals. There is no information related to the mass production of these products and, therefore, no information is available on their deterioration kinetics.

Empirical mathematical models related to the kinetics of degradation of physicochemical, microbiological, and sensory properties are widely used in the food industry because an approximation can be made about the behavior of a given food matrix during its storage to establish the shelf-life of certain foods [4]. The storage temperature control is another key variable to determine the kinetics of deterioration of food, which is why the Arrhenius theoretical model allows a better approach towards understanding the degradation reactions that occur within a food system because it involves changes in storage temperature with the equilibrium constants of the reactions involved [5].

The main objective of this work was to model mathematically, by means of empirical equations, the degradation during its period of storage of a formulation of a food product derived from blackberry with functional characteristics and determine the shelf-life.

# **Materials and Methods**

The blackberries were purchased at the wholesale supply center for fruits and vegetables in the city of Armenia, Quindío, Colombia in a state of maturation between 4 and 5, which were harvested in the population of Salento, Quindío.

The plant material was taken to the pilot plant in University of Quindío, located in Armenia, Quindío, Colombia where these were washed, selected, blanched, and homogenized in a conventional blender. The homogenate was passed through a 100- $\mu$ m pore size mesh to remove large residues, then portioned into 200-g portions and frozen for later use [6].

Subsequently, the portions were thawed and pectinase enzyme was added to reduce their viscosity; Xanthan gums and carboxymethyl cellulose to stabilize the pulp; sodium citrate as acidity regulator; sucralose as a non-caloric sweetener; and calcium citrate and ascorbic acid as physiologically active components [7]. The percentages of each of the ingredients are reported in Table 1. Thereafter, the pulp obtained was subjected to pasteurization for 30 s at 80 °C to, then, perform the physicochemical and sensory analyses of its deterioration. This formulation was made based on the Colombian technical standard 5468 "juices, nectars, purées (pulps), and fruit concentrates" and Resolution 333 of 2011 by the Ministry of Social Protection "by which the technical regulation is established on the requirements of labeling or nutritional labeling that must be met for food packaged for human consumption".

Component	Percentage %
Pulp	98.716
Enzime (Pectinase)	0.076
Gum (CMC)	0.05
Sodium citrate	0.25
Calcium (20% VDR) Calcium citrate	0.41
Vitamin C (20% VDR) Ascorbic acid	0.004
Sucralose (BPM)	0.5
TOTAL	100

 
 Table I. Composition of the blackberry pulp formulation fortified with calcium and vitamin C.

#### **Evaluation of deterioration kinetics.**

The functional pulp obtained was stored at three temperatures (15, 25, and 35 °C) and at constant relative humidity of 80% for 20 days. Every three days, starting from day zero, the physicochemical characterization was carried out by analyzing the pH variables via the potentiometric method, using a glass electrode, according to the AOAC 981.12 method (AOAC 1980); viscosity was measured with a rotational viscometer (Selecta, reference ST 2010) at room temperature; color was measured with a Minolta CR 10 spectrophotometer, with D65 illuminant and 10° standard observer; the ICD-L\* a\* b\* coordinates were determined from the reflection spectra of the samples, where the luminance component (L \*) varies between 0 and 100 and the a\* component (greenred axis) and b\* component (blue-yellow axis) can be between +127 and -128 [8]; soluble solids were determined with a THERMO table refractometer, scale from 0 to 85 <sup>o</sup>Brix, following the AOAC 932.12 method (AOAC 1980). Microbiological analysis was performed on the pulp by determining mesophilic and total coliform microorganisms. The mesophilic microorganism count was performed by serial dilutions of fruit pulp up to 10-3 in peptone water and coliform counts were performed by using the most-probable number technique [9].

Prior to the physicochemical analyses, a sensory analysis was performed on samples stored at 25 °C. The samples were introduced at random and simultaneously during the storage period to the previously semi-drilled judges, who were 10 students from the Food Engineering Program at Universidad del Quindío and who had previous knowledge about sensory analysis. The descriptors of fla-

vor, color, aroma, texture, and general appearance were evaluated on a scale of 1 to 5 where 1 is very unpleasant and 5 I like a lot, admitting that values below 3 are unacceptable parameters for consumption, that is, values rejecting the quality parameter. An analysis of variance was performed to determine the variable most affected during storage in order to model the deterioration kinetics with a 95% confidence level [10, 11].

The mathematical modeling that describes the degradation of the blackberry pulp was made based on the integration of the kinetics and Arrhenius (1 to 3) proposed by Palazon et al., 2009 [4].

detected at 11 days. Therefore, on these days the sensory

$$Q = Qo - kt \tag{1}$$

$$Q = Qoe^{-kt}$$
  

$$k = koe^{-Ea/RT}$$
(2)

$$Q = Qo - kote^{-E\alpha/RT}$$
(3)

 $Q = Qoe^{-kote^{-Ea/RT}}$ Source: Palazon et al., 2009 [4]

Where:

Q = value of the physicochemical variable evaluated at time t

Qo = Value of the physicochemical variable evaluated at the initial time.

 $\mathbf{k} =$  equilibrium constant of the deterioration reaction

ko = Preexponential constant.

T = Time in days of storage.

Ea = Activation energy of the deterioration reaction

T = temperature in Kelvin.

R = Gas constant.

The results were proceesed with the Statgraphisc Centurion 15.2 software.

#### **Results and Discussion**

The final time of the analyses was determined by the appearance of mesophilic microorganisms, which for samples stored at 15 °C were detected at 21 days; for samples stored at 25 °C, these were observed at 18 days; and for samples storage at 35 °C, the microorganisms

and physicochemical analyses were not conducted and, therefore, the end point of the kinetics was taken the day before detecting the mesophilic microorganisms, observing that at 15 °C the analyses were carried out until day 18; at 25 °C until day 15; and at 15 °C until day 10. Total aerobic microorganisms were not detected at any stage of kinetics.

Similar research on the shelf-life of canteloupe reported the occurrence of mesophilic microorganisms at 7 days of storage at room temperature [12]. Other researchers reported the occurrence of mesophilic microorganisms in carrot juice treated with ultrasound at 20 days of storage [13].

Tables 2, 3, and 4 show the zero order and first order kinetic equations for the physicochemical variables evaluated at the three working temperatures. The equations were obtained by analyzing the product 20 days for samples stored at 15 °C, 16 days for those stored at 25 °C, and 10 days for 35 °C. Completion times were determined when the product is totally degraded physicochemically and microbiologically.

It can be observed in the kinetic equations obtained and according to the results of the analysis of variance that the parameter most influenced during the storage is that of the a\* chromatic axis, which in all the working temperatures and the kinetic models calculated obtained a p-value of 0.0000. This indicates statistical significance in its variability during the product's storage time. Given that all the kinetic equations calculated for this variable have a positive slope, this indicates that the violet-purple color of the blackberries is lost and becomes increasingly red, which is reflected in the increase of the a\* parameter with the weather. This variability was also detected in the sensory analysis performed because the kinetic equation for the aroma descriptor also had a high significance (Table 7). This degradation is mainly due to the gradual decomposition that anthocyanins undergo due to thermal exposure, light of the medium, or pH changes [14-16]. In other works, mathematical models for the degradation of mango fruit have been proposed [6], where the authors propose nonlinear mathematical models to describe the behavior of mango during storage.

In terms of viscosity, it was observed that in spite of the stabilization process carried out on the blackberry pulp,

the water tends to be strongly retained in the polysaccharides of the pulp matrix generating increased viscosity with increased time [16-18]. This indicates that the initial formulation has failed and that further studies may be needed to improve the stabilization of the product for the viscosity to remain constant over time.

Previous studies have reported kinetic empirical models to describe food degradation. For example, some authors proposed zero-order and first-order empirical kinetic equations to determine the shelf-life of bananas treated with soy lecithin [19]; other authors empirically modeled the physicochemical behavior of compotes based on various fruits to know their useful life [20].

Table 5 presents the empirical equations of the Arrhenius model. It can be observed that the only variable that does not follow this model is a\* because its slope is positive and, therefore, has a positive correlation coefficient. One of the main features of the Arrhenius model is that its slope must be negative given that the temperature inverse must always be inversely proportional to the logarithm of the equilibrium constant of the reaction. This could lead to the lack of color homogeneity of the pulps worked, which leads to not all the pulps having the same color at the time of the analysis and, therefore, their changes during storage could be different. The other variables behaved close to the theoretical model proposed by Arrhenius because their correlation coefficients were close to -1, indicating an inverse linear variability between the temperature inverse and the logarithm of their equilibrium constants. Other authors have reported results of Arrhenius mathematical models applied to the degradation of food systems, like formulations for children [20], patulin in applesauce [21], acerola pulp [14], and orange juice [15].

Table 6 shows the coefficients of the empirical equations obtained by integrating the kinetic equations with those by Arrhenius (3). With these equations, the variability of each of the parameters analyzed can be mathematically related by linkinthe storage time and the storage temperature to the desired quality factor of the product.

Knowing these two parameters can predict the behavior of each of the variables analyzed and can, therefore, be an approximation to determine the useful life of the product.

The correlation coefficients of each of the equations in Table 6 indicate that the only variable that does not follow the

	Zero order			First order			
	Equation	R	p value	Equation	R	p value	
L*	L* = 30.9857 + 0.0952381*Day	0.611942	0.0032	Log L* = 1.49135 + 0.00127547* Day	0.606521	0.0036	
a*	a* = 13.6881 - 0.192857* Day	-0.802404	0.0000	Log a* = 1.13887 - 0.00718728* Day	-0.802234	0.0000	
b*	b* = 8.58452 - 0.0384921* Day	-0.164707	0.4756	Log b* = 0.920609 - 0.00103555* Day	-0.107255	0.6435	
pH	pH = 4.36893 + 0.0115476* Day	0.632453	0.0021	log pH = 0.640207 + 0.0011384* Day	0.632678	0.0021	
Brix	Brix = 8.37262 - 0.0107143* Day	-0.190235	0.4088	Log Brix = 0.921998 - 0.000502043* Day	-0.173851	0.4510	
U	u = 402.655 + 10.5992* Day	0.72546	0.0002	Log u = 2.59443 + 0.0105167*Day	0.711008	0.0003	

Table II. Kinetic equations of deterioration of blackberry pulp at 15 °C.

Q	Zero order			First order			
	Equation	R	p value	Equation	R	p value	
L*	L* = 29.4892 + 0.89018*Day	0.475516	0.0732	Log L* = 1.53249 + 0.0027165* Day	0.842599	0.0001	
a*	a* = 13.7467 + 0.273333* Day	0.914176	0.0000	Log a* = 1.13874 + 0.00781667* Day	0.912417	0.0000	
b*	b* = 8.7434 - 0.0277778* Day	-0.35540	0.1936	Log b* = 0.940896 - 0.00134492* Day	-0.340591	0.2142	
pН	pH = 4.45 + 0.0121111* Day	0.761512	0.0010	log pH = 0.648336 + 0.00116834* Day	0.761168	0.0010	
Brix	Brix = 6.42 + 0.0366667* Day	0.260823	0.3478	Log Brix = 0.804143 + 0.00267469* Day	0.3385	0.3385	
U	u = 671.467 + 37.8889* Day	0.836354	0.0001	Log u = 2.81413 + 0.0211285* Day	0.7979	0.0004	

Table III. Kinetic equations of deterioration of blackberry pulp at 25 °C.

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	Zero order			First order			
	Equation	R	p value	Equation	R	p value	
L*	L* = 31.9619 + 0.113968*Day	0.610526	0.0071	Log L* = 1.50474 + 0.00148981* Day	0.605653	0.0077	
a*	a* = 13.2857 - 0.444762* Day	-0.976074	0.0000	Log a*= 1.13507 - 0.0199557* Day	-0.972451	0.0000	
b*	b* = 7.6254 + 0.0292063* Day	0.345092	0.1608	Log b* = 0.882183 + 0.00156047* Day	0.32848	0.1832	
pН	pH = 4.2877 + 0.0195556* Day	0.682562	0.0018	log pH = 0.632053 + 0.00193894* Day	0.677708	0.0020	
Brix	Brix = 8.312 + 0.0901587* Day	0.740847	0.0004	Log Brix = 0.92096 +0.0042312* Day	0.738899	0.0005	
U	u = 465.175 + 18.0508* Day	0.754473	0.0003	Log u = 2.65648 + 0.0148511* Day	0.756417	0.0003	

Table IV. Kinetic equations of deterioration of blackberry pulp at 35 °C

The correlation coefficients of each of the equations in Table 6 indicate that the only variable that does not follow the proposed empirical model is the a\* chromatic axis; this may be due to the fact that in the Arrhenius model there was also no correlation between the variables of temperature and a\* chromatic axis, and the u variable, although it has a negative correlation coefficient, its value is much less than -1, indicating a low linear correlation between temperature and time against the product's viscosity. The other variables have correlation coefficients in agreement with the empirical model, which suggests that they could be used at some point to predict the shelf-life times of the blackberry pulp at different temperatures and storage times.

The activation energies of the degradation reactions are reported in Table 6. High activation energy can be observed for viscosity. This variable is directly related with the hydrolysis that can be encountered by the polysaccharides of high molecular weight that are in the food matrix and that cause water retention. This high value indicates the low rate that this hydrolysis process has inside the pulp [9].

The high values of activation energy indicate a high association of the process indicated with temperature, that is to say, at very high values of activation energy, the reaction will be carried out much faster at high temperatures and, therefore, at low values of activation energy

the reaction will take much slower at low temperatures. This is in close agreement with the fact that all fluids by increasing their temperature rapidly decrease their viscosity, causing a much faster change [14]. Other ac tivation energy values for the study of food degradation have been reported: Remini et al., [5] reported activation energies for global color degradation ( $\Delta E$ ) in orange pulp between 49 and 99 kJ/mol; Mercali et al., [14] reported

values of 71.79 and 71.94 kJ/mol for the activation energy of the degradation reaction of anthocyanins in acerola pulp. In turn, Palazón et al., [4] published activation energy values in kJ/mol for the color degradation of an apple compote for a\* at 13.5, b\* at 15.6, and for L\* at 16.4.

Equ	ation	for	variable		r
Log 1058	a* 3.11*1	= l/T	-3.67479	+	0.346221
Log 297.	<b>b*</b> 849*1	= l/T	0.98789	-	- 0.910023
Log 446.	brix 921*1	: = l/T	1.53957	-	- 0.937731
Log 160.	L* 945*1	= l/T	0.906953	-	- 0.997034
Log 35.1	рН 626*1	= l/T	0.132489	-	- 0.860254
Log 3537	u 73.8*1	= l/T	140.973	-	- 0.309842

Table 5. Arrhenius equation of pulp deterioration kinetics.

The correlation coefficients of each of the equations in Table 6 indicate that the only variable that does not follow the proposed empirical model is the a\* chromatic axis; this may be due to the fact that in the Arrhenius model there was also no correlation between the variables of temperature and a\* chromatic axis, and the u variable, although it has a negative correlation coefficient, its value is much less than -1, indicating a low linear correlation between temperature and time against the product's viscosity. The other variables have correlation coefficients in agreement with the empirical model, which suggests that they could be used at some point to predict the shelf-life times of the blackberry pulp at different temperatures and storage times. The activation energies of the degradation reactions are reported in Table 6. High activation energy can be observed for viscosity. This variable is directly related with the hydrolysis that can be encountered by the polysaccharides of high molecular weight that are in the food matrix and that cause water retention. This high value indicates the low rate that this hydrolysis process has inside the pulp [9].

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Variable	00	Ко	Ea (KJ/mol)	r
a*	13.7390	0.00021145	-8.7975497	0.327465
b*	8.4580	9.72500873	2.47643573	- 0.922915
brix	8.2194	34.6393712	3.71587996	- 0.998524
L*	30.6701	8.07147675	1.33816111	- 0.040004
pН	4.3006	1.35671617	0.29235592	- 0.887046
U	453.75	9.397E+14	294.111923	- 0.282767

 
 Table VI. Integrated equation for the degradation kinetics of blackberry pulp.
 Table 7 presents the kinetic equations for the descriptors used in the sensory analysis of blackberry pulp stored at 25 °C. The equations were obtained by performing the analyses to the final point of rejection of the appearance by the judges.

It can be observed that the variables of appearance, color, and taste follow zero-order kinetics, whereas aroma and texture are modeled with first-order kinetics. It can also be observed that the aroma variable has a correlation coefficient far from -1, which indicates the low correlation between this variable and the storage time with which it can be confirmed that aroma is slightly affected during storage and, therefore, it is not a variable to take into account to determine the shelf-life of the product. The variables of appearance and color are the most affected during storage, according to the sensory analysis, given that the analysis of variance shows a p value of 0.000, indicating a high significance of these parameters during storage.

Variables	Equation	P Value	R	Order
Appearance (App)	App = 4.5714 - 0.1873*t	0.0000	- 0.8225	Zero
Color (Co)	Co = 4.4761 - 0.1746* t	0.0000	- 0.8385	Zero
Flavor (Fl)	Fl = 4.2539 - 0.1079* t	0.0006	- 0.7259	Zero
Texture (Te)	Log te= 0.6641 - 0.0181* t	0.0003	- 0.7601	First

Table VII. Kinetic equations of the sensory attributes of blackberry pulp stored at 25  $^{\rm o}{\rm C}$ 

Some authors have reported first-order kinetic empirical equations for sensory evaluation of color degradation in pasteurized strawberry juices for 30 days stored at 15 °C [22] and others have evaluated the shelf-life of pasteurized orange juice by kinetic equations, reporting a product rejection time at 341 days stored at 25 °C [23].

Figure 1 shows the evaluations carried out by the judges throughout the storage period. For appearance, texture,

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Medias y95,0% Intervalos LSD

and color, there is statistical difference between days 12 to 15, which is due to the viscosity and color changes detected in the physicochemical analyses, that is, the viscosity changes because increased water trapped by the pulp polysaccharides allowed an increase in the viscosity and, therefore, an immediate detection by part of the judges in the sensory evaluation. For color, the perception of the judges goes hand in hand with the physicochemical analysis, reaffirming the degradation that anthocyanins suffer during storage by altering the a\* chromatic axis and causing a change in the visual perception of the product.

For aroma, no significant differences were reported during the whole evaluation; therefore, it is not a variable to be considered during storage. Color begins to change significantly from day 9 of storage; henceforth, the judges did not assess satisfactorily the color of the samples. Taste and texture change significantly from day 12 of storage at 25 °C.

The information provided by the sensory evaluation can be corroborated because it agrees with the results obtained in the physicochemical analyses, which helps to determine the shelf-life times of the product.

Medias y 95,0% Intervalos LSD



Día

5,5

4,5

3,5

2,5

1.5

0

3

6

9

12

15

Textura



Medias y 95,0% Intervalos LSD

Figure 1. Sensory behavior of blackberry pulp during storage.

#### Conclusion

The blackberry formulation was evaluated until the detection of mesophilic microorganisms, which were detected on day 21 of storage at 15 °C, at day 18 at 25 °C, and at day 11 at 35 °C. The empirical kinetic equations for the variables L\*, a\*, b\*, brix, pH, and viscosity were considered for each working temperature.

The parameters of the Arrhenius theoretical model were calculated for each variable and, in turn, these two equations were integrated to obtain an integrated model that represents the degradation of the blackberry pulp by integrating temperature and time within the process variables.

A sensory analysis was performed and the correlation with the physicochemical data obtained could be determined, given that the rejection times by the panelists were very close to those obtained in the physicochemical and microbiological tests.

It was possible to determine sensorily that the product was rejected on average for all descriptors at 12 days.

All the results of the sensory analysis agree faithfully with the results of the physicochemical analysis, corro borating and reaffirming the calculation of the shelf-life times determined in this work for the functional blackberry pulp.

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