










Predicting the Shelf Life of Cup Chocolate Using the Arrhenius Model Based on Peroxide Value

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ABSTRACT

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The shelf life of a food product is a limited period of time after production and packaging, during which it maintains the necessary and acceptable level of quality for final consumption. The aim of the research was to predict the shelf life of chocolate packaged in two bilaminated containers with respect to peroxide value, using accelerated testing and constant relative humidity. The peroxide value was evaluated by potentiometric titration. The order of the reaction was defined and with the Arrhenius model the degradation rate constant was found for each container and temperature of study. Shelf life was determined with the kinetic equation of oxidation compound formation at 5, 20 and 35°C at 217, 114 and 64 days for the 20 microns (μ) packages, and 114, 95 and 81 days for the 50 μ packages respectively. It is concluded that the 20 μ packaging between a storage temperature of 15 and 18°C is the suggested packaging with a shelf life between 141 and 124 days. The results would enable those working in the chocolate manufacturing and packaging industry to take the temperatures and types of packaging studied as a reference in their production.

1. INTRODUCTION

Cocoa (*Theobroma cacao*) is a fruit of tropical origin, found in Peru, Ecuador, Bolivia, Colombia and Venezuela [1-3]. It was also cultivated by indigenous Mexican peoples and later spread to Asia, Africa, Oceania and the Caribbean [4]. There are three types of cocoa varieties: criollo, forastero and trinitario [5-7]. The physical characteristics vary between types, with fruits showing a great diversity of shapes, textures and colours in both the unripe and ripe stages. In addition, the fruits of Criollo type cocoa have fewer total and viable seeds per ear, while the fruits of Amazonian forastero and Trinitario type cocoa do not differ statistically with respect to these characteristics [8]. Due to its importance and preference, it is one of the most demanded products at industrial level, where Peru stands out as one of the pioneers in production and exportation [9]. Among the parameters influencing the selection of a particular type of cocoa-by-cocoa manufacturers are cocoa type by chocolate manufacturers include physical aspects such as chocolate, include physical aspects such as bean size, shell bean size, shell percentage, fat content, butter hardness and moisture, fat content, butter hardness and moisture content. For this reason, it is important to evaluate

these quality parameters in criollo cocoa beans which are of great interest to chocolatiers, due to the organoleptic the organoleptic attributes they contain, allowing to control and eliminate control and eliminate off-flavours caused by moulds, smoke moulds, smoke, acidity and astringency, which are the result of the conditioning the result of the conditioning factors of the quality of the almonds during post-harvesting [10]. The most important derivative of cocoa is chocolate, which was discovered by Aztec and Inca tribes, who prepared cocoa beans as a cold drink [11]. Chocolate is commonly consumed for its nutritional quality and palatability, and is classified according to the percentage of cocoa mass [12]. In turn, the use of 100% cocoa for the production of cup chocolate offers commercial advantage for local cocoa producers, due to a global trend towards the consumption of "organic" and "natural" foods [13].

To obtain a perennial and quality chocolate, there are multiple factors that influence its stability, so a bad manufacturing practice would have a detrimental influence on the final product, this action can be caused from the collection of raw material to the processing stage [14]. However, the quality of the chocolate depends on the cocoa variety, the chemical composition of the beans, the specific content of

stored proteins and the polysaccharides and polyphenols that determine the type and amount of precursors formed during the fermentation and drying process, leading to the formation of specific chocolate aromas in the subsequent roasting and conching processes [15, 16]. In the manufacturing process, the unitary operations of conching and tempering influence and determine the quality of the product [17]. Conching defines the texture, rheology and chemical changes such as acidity decrease, odour formation and desirable flavours in chocolate (generated by the conversion of precursors formed during fermentation and roasting) [18]. It also allows the elimination of moisture and volatile acids, obtaining a perfect emulsion, contributing to the quality and smoothness of the product [19]. Packaging can promote anaerobic conditions or modify the atmosphere between the food and the packaging material, so that under such conditions the shelf life of the food can be prolonged [20]. The shelf life of chocolate depends on processing, storage, relative humidity, oxygen, temperature and packaging [21], where quality loss is generally associated with fat oxidation that occurs by a free radical propagation reaction, in which peroxides and hydroperoxides responsible for oxidative rancidity are formed from fatty acids and oxygen [22]. In addition, fat efflorescence on the surface of chocolate occurs, which are whitish cocoa butter crystals called fat bloom [23], generated by phase separation due to high and low melting point triglycerides and polymorphic fat transformation, caused by inadequate storage (above 20°C) and relative humidity (above 60%), which decreases the sensory quality of chocolate, although it is still safe for human health [24].

Shelf-life is a finite period after manufacturing and packaging, during which the food retains a level of safety, required and acceptable quality for consumption [25]. Quantification is a fundamental factor [26], as it is part of the development of new products and packaging or formulation changes, given its importance in food safety and quality, which is a constant for producers and consumers. The peroxide value (PV) is an indicator that can be used as a predictor of quality during the secondary shelf life of chocolate, throughout its storage it should not exceed 10 meq active O₂/kg (milliequivalents of active oxygen per kilogramme) fat or oil, as established by the AOAC 2003 peroxide value determination [27].

2. RESEARCH METHODOLOGY

The general objective of the study was to predict the shelf life of chocolate by means of accelerated tests based on the peroxide value as a quality descriptor; the specific objectives were: to evaluate the peroxide value of chocolate packaged in 20 and 50 μ bilaminated aluminium foil, stored at 5, 20 and 35°C; to apply the Arrhenius equation to determine the chocolate degradation rate constant based on the peroxide value; and to determine the shelf life of chocolate.

The cup chocolate was obtained from the production of the company Agrotec, from the province of Jaen, Cajamarca, Peru, in the presentation of 100g, with 100% cocoa, packaged in 20μ and 50μ bi laminated aluminum foil. Its packaging is manufactured by alloying aluminium (Al) plus iron (Fe) to give the foil strength and therefore requires high processing strength to obtain an adequate lamination; therefore, the greater the number and size of Al-Fe intermetallic phases, the

greater the risk of micro-perforations in the aluminium foil structure [28]. The sample consisted of 24 tablets, 12 packaged in 20μ bi laminated aluminum foil and 12 in 50μ bi laminated aluminum foil, duly labeled, these were stored at three temperatures: refrigeration 5°C, ambient 20°C and oven 35°C, with a relative humidity of 65%. Evaluations were carried out every 5 days during 15 days of storage. Non-probabilistic convenience sampling was used to arbitrarily select study participants according to the accelerated testing and considering the reduction of contaminating reagents [29].

The study makes use of the ASLT, where three acceleration temperatures are considered 5, 20 y 35°C; chocolate is stored between 15 and 18°C as the recommended natural condition, temperatures of 20 and 35°C are considered as fat oxidation acceleration conditions, also 5°C is set as the storage acceleration condition for chocolate, because a sudden temperature variation causes the appearance of fat bloom in a premature time [24, 30, 31], also accelerates the rate of auto-oxidation and reaction mechanisms [32]. However, humidities above 75% cause this phenomenon to appear, which in sugar chocolates is called sugar bloom [30].

The PV was determined for each sample by potentiometric titration using NTP 209.006:2013 Edible oils and fats, and the soxhlet method NTP 209.263:2013 was used to extract the fat from the chocolate.

The estimation of the useful life was carried out using accelerated tests, the data obtained were systematized in a data matrix, and the coefficient of determination was calculated by means of linear regression (R^2) using rstudio software, with which the reaction order of the decay kinetics for the formation of undesirable compounds was determined between zero or one. Once the order of the reaction was obtained, a second regression was plotted using the slopes and temperature. Consequently, the second reaction was adjusted to the linearized Arrhenius model, from which the activation energy is obtained (Ea) and the pre-exponential factor (K_0), variables that help to find the rate of decay constant (K), see Eqs. (1)-(2) [33].

$$K = K_0 e^{-\left(\frac{Ea}{RT}\right)} \quad (1)$$

$$\ln K = -\left(\frac{Ea}{R}\right)\frac{1}{T} + \ln[K_0] \quad (2)$$

where:

T =absolute temperature

R =Universal gas constant (1.986 cal/mol °K)

Finalizing with the replacement of the decay rate in Eq. (3) and Eq. (4) of the decay kinetics of order zero or one according to the result for formation of undesirable compounds.

$$\text{Order 0 } t = \frac{A_0 + A}{K} \quad (3)$$

$$\text{Order 1 } t = \frac{\ln A_0 + \ln A}{K} \quad (4)$$

where, t is the useful life time, K is the decay kinetic constant, A_0 is the initial PV value of the chocolate. However, chocolate was considered not to be within the permissible limits if the PV value is greater than 10 meq active O₂/kg fat and this value is assumed to be the limit of acceptability $A=10$ [34].

3. RESULTS

3.1 Determination of peroxide value

3.1.1 Initial peroxide value

Chocolate samples in 20 μ and 50 μ bi laminated packaging in the initial condition for the three treatments at 5 $^{\circ}$ C, 20 $^{\circ}$ C and 35 $^{\circ}$ C obtained a PV of 1.116 and 1.184 meq active O₂/kg fat, respectively.

3.1.2 Peroxide value results after ASLT

The results of the simulation of storage of packaged chocolate at various temperatures with increasing PV are presented in Figure 1.

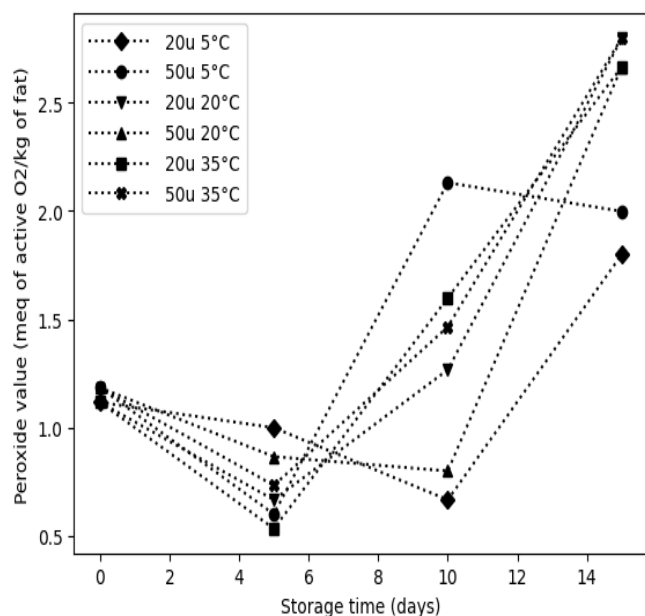


Figure 1. Means of peroxide value of the peroxide value of cup chocolate

Figure 2 shows the multiple box-plot graphs where the dispersion of the peroxide index data set, evaluated at three temperatures (5, 20 and 35 $^{\circ}$ C) with two types of packaging (20 and 50 μ bilaminate), is analyzed graphically. It is observed, that the 20 μ aluminum foil packing at 5 $^{\circ}$ C presents better symmetry of its data, and the extension of the whiskers are

shorter, but outlier data are perceived, one for each upper and lower limit; however, at 20 and 35 $^{\circ}$ C they show greater asymmetry to the right side of the boxes with greater extension of the whisker in the upper limit without presence of outlier data. However, for the 50 μ packing at 5 $^{\circ}$ C the data in the box present asymmetry to the right side, with greater extension of their whiskers and showing an outlier data at the upper limit. The 20 $^{\circ}$ C temperature shows a greater distribution of data on the left side, but less extension in the whiskers and with two outliers at the upper limit. Regarding the temperature of 35 $^{\circ}$ C, there is symmetry on the right side with greater extension in the upper limit and with one outlier in the upper limit and one in the lower limit.

In summary, to analyze the peroxide value in the oxidation of chocolate fat tends to be very variable and unstable as evidenced in Figure 2, where there are outliers, asymmetry and symmetry of the data in the boxes and instability in the extension of the whiskers of the upper and lower limit.

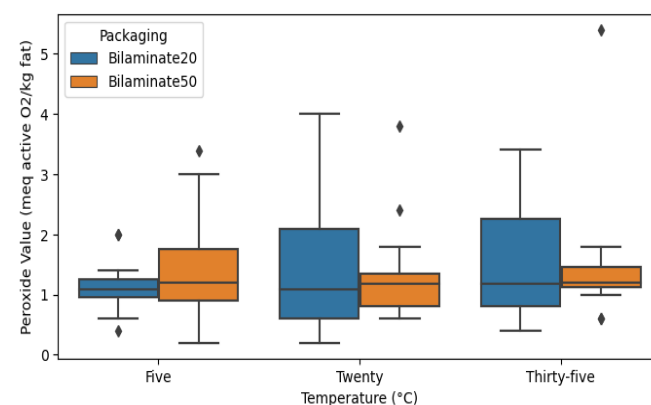


Figure 2. Peroxide value vs. temperature and packaging type

3.2 Estimation of the shelf life of cup chocolate

3.2.1 Reaction order

By obtaining the PV increment data for each storage temperature and packaging materials (Table 1); the chemical kinetics reaction order of undesirable compound formation, defined as zero order, was determined by the highest value of R² obtained from the tabulation of storage time versus peroxide value data for order zero and one, as shown in Table 2.

Table 1. Determining the order of the reaction

Descriptor	T $^{\circ}$ C	Presentation	Reaction Order	Linear Regression	R ²	Selection
Peroxide value	5	Bilaminate 20 μ	0	y=0.0343x+0.8879	0.2163	0
			1	y=0.0205x-0.0815	0.1049	
		Bilaminate 50 μ	0	y=0.0795x+0.8819	0.5081	
			1	y=0.0568x-0.1493	0.3892	
	20	Bilaminate 20 μ	0	y=0.1128x+0.6152	0.6186	0
			1	y=0.0679x-0.2675	0.5467	
		Bilaminate 50 μ	0	y=0.0875x+0.7219	0.418	
			1	y=0.0471x-0.1582	0.3053	
	35	Bilaminate 20 μ	0	y=0.1142x+0.6214	0.6665	0
			1	y=0.0742x-0.3238	0.5011	
		Bilaminate 50 μ	0	y=0.1116x+0.7083	0.6568	
			1	y=0.0655x-0.174	0.5781	

3.2.2 Activation energy and pre-exponential factor

From the regression between the inverse of the temperature and the slope value of each first order plot, the value of lnK

and 1/T was plotted in a semi-logarithmic plot for each container (Figures 3 and 4), which was fitted with the linearized Arrhenius model, Eq. (2), where lnK is the ordinate

and $1/T$ is the abscissa, obtaining the value of E_a (Table 2) and K_0 (Table 3) for each type of container.

Table 2. Values of $-E_a/R$ and E_a for each package

Bilaminated Packaging	$-E_a/R$	R (cal/mol $^{\circ}K$)	E_a (cal/mol $^{\circ}K$)
20 μ	-3495	1.986	6941.07
50 μ	-961	1.986	1908.55

Table 3. Values of $\ln K_0$ y K_0 for each type of packaging

Bilaminated Packaging	$\ln K_0$	K_0
20 μ	9.3678	11705.34
50 μ	0.8967	2.4515

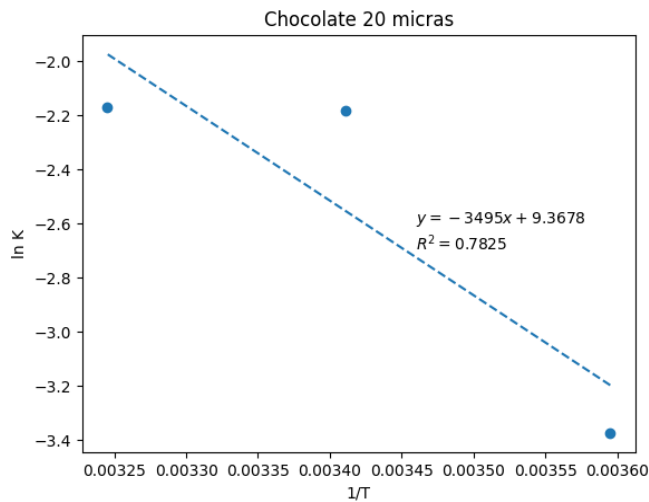


Figure 3. Relationship between $\ln K$ with $1/T$ of chocolate for 20 μ packaging

3.2.3 Coefficient of decay kinetics and useful life

The values of K for each temperature and container of the experiment (Table 4) were determined with the E_a and K_0 adjusted to Eq. (2). Consequently, the estimated chocolate shelf life for each container (Table 5) was determined by using the zero-order kinetics equation for compound formation, Eq. (4), where the acceptability limit is 10.

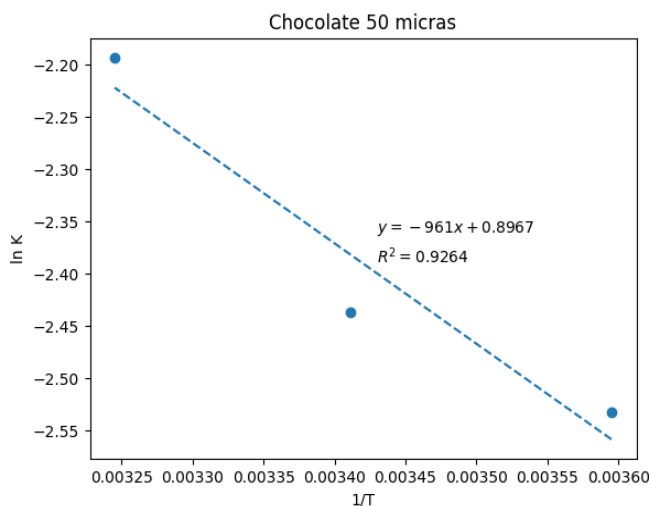


Figure 4. Relationship between $\ln K$ with $1/T$ of chocolate for 50 μ packaging

Table 4. K values and shelf life for each temperature and type of container in the experiment

Packaging	Storage Temperature $^{\circ}C$ or K	K (days $^{-1}$)	t (days)
Bi laminate 20 μ	5 or 278.15	0.04087	217
	20 or 293.15	0.07774	114
	35 or 308.15	0.13889	64
Bi laminate 50 μ	5 or 278.15	0.07744	114
	20 or 293.15	0.09241	95
	35 or 308.15	0.10840	81

Table 5. K -values and shelf life for storage temperatures

Packaging	Storage Temperature $^{\circ}C$ or K	K (days $^{-1}$)	t (days)
Bi laminate 20 μ	0 or 273.15	0.03247	274
	10 or 283.15	0.05102	174
	15 or 288.15	0.06321	141
	25 or 298.15	0.09494	94
	30 or 303.15	0.11519	77
Bi laminate 50 μ	0 or 273.15	0.07269	121
	10 or 283.15	0.08231	107
	15 or 288.15	0.08730	101
	25 or 298.15	0.09764	90
	30 or 303.15	0.10297	85

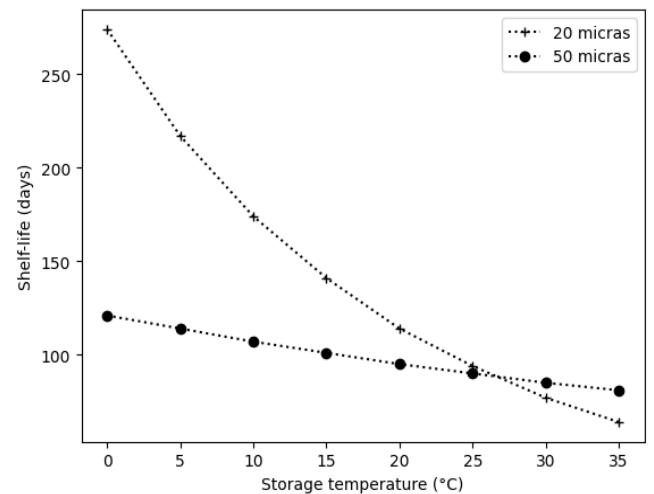


Figure 5. Shelf life of specialty coffees

4. DISCUSSION

The PV values after storage for cup chocolate packaged in 20 μ and 50 μ are 1.8 and 2.0 meq O_2 active/Kg fat for 5 $^{\circ}C$, 2.8 and 2.7 meq O_2 active/Kg fat for 20 $^{\circ}C$ and 2.7 and 2.8 meq O_2 active/Kg fat for 35 $^{\circ}C$ respectively, results that show a non-linear increasing trend of PV for the two packages. These values are similar to the results obtained in the research with 5 types of chocolates stored at 20 $^{\circ}C$, 25 $^{\circ}C$ and 30 $^{\circ}C$ for 56, 42 and 30 days, respectively, where the results showed a non-linear trend of increase of the PI, with fluctuations in the values of the PV values [35]. However, in the results reported for cup chocolate stored for 90 days with a RH of 80%, packaged in mono-oriented polypropylene and polyester aluminum with polyethylene barrier, there is a difference because a linear trend of increasing peroxide index is observed for the 2

packages, being the final PV values lower than 4.1 and 4.23 meq O₂ active/Kg fat for 20°C, 4.72 and 4.49 meq O₂ active/Kg fat for 25°C and 4.79 and 4.57 meq O₂ active/Kg fat for 30°C respectively [36].

The PV is related to the extraction of the fat and the distribution in the geometry of the chocolate bar, there is also a dependence on the chocolate concentration, the factors involved in the process also influence the non-homogeneity and non-linearity of the result of the analysis. It can also be related to the stability of the product at the time of packaging.

It would serve as a benchmark for chocolate manufacturers to estimate how long their product maintains a certain quality and this factor would allow them to improve storage, production and marketing processes.

With regard to the kinetics of fat oxidation, the behavior reported by Tolve et al. [22] on the formation of hydroxyperoxides in spreadable chocolate creams as a function of storage temperature is nonlinear and follows a kinetic of order one, with a growth trend, but with some values decreasing with increasing temperature, an oxidative phenomenon similar to the one obtained in the research, unlike the kinetic that turned out to be of order zero, an oxidative phenomenon of fat degradation that deserves further study. In other words, the oxidative phenomenon of chocolates under variable storage conditions should be studied by quantifying the formation and concentration of primary and secondary oxidation compounds called peroxides and hydroperoxides. This information will allow predicting the behaviour of the oxidation phenomenon of chocolates.

In research with cup chocolate packaged in mono-oriented polypropylene and polyester aluminum with polyethylene barrier, an estimated shelf life of 135 and 148 days was reported for storage at 18°C, respectively [36]; also for sugar-free rate chocolate packaged in bioriented polypropylene, they estimated the shelf life for storage at 18°C with a relative humidity of 80% to be 255.87 days [21], furthermore, the shelf life of instant chocolate vacuum-packed in polypropylene plastics by the ASLT method was reported to be 281, 58, 240, 99 and 113 days at 30°C, 40°C and 50°C, respectively [38], estimated times that differ from the results of the research (shorter shelf life), where an estimated shelf life at 18°C of 124 and 98 days is obtained for the 20 μ and 50 μ bi laminated aluminum foil packings, respectively. However, they estimated the shelf life of chocolate spreads at 20°C to be 59.5 days [22], less than the estimated time in the research (Figure 5). The difference in the estimated shelf-life research may depend on the model used, because the reference authors use a multivariate method with several quality descriptors (sensory, physical-chemical and microbiological analysis) and also controlled storage conditions.

5. CONCLUSIONS

Shelf life of 100% cocoa cup chocolate in 20 μ sachets and 50 μ bi laminated aluminum foil packaging was estimated by mathematical modeling using the Arrhenius equation and zero-order chemical reaction kinetics for the formation of undesirable compounds, stored under accelerated conditions for 15 days at 5°C, 20°C and 35°C and 65% RH, being 217, 114 and 64 days for the 20 μ bi laminated packaging, and 114, 95 and 81 days for the 50 μ packaging, respectively. Mathematical models could be developed, which estimate the variability of the peroxide value with respect to its stability.

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