Scientific Bulletin. Series F. Biotechnologies, Vol. XXV, No. 1, 2021
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

COMPARATIVE ANALYSIS OF ESTIMATED SHELF LIFE, APPROACHING ACCELERATED AGING METHODS

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Abstract

The aim of the research was to compare the shelf life of fruit smoothies, obtained by two different accelerated aging methods. An accelerated shelf life test (ASLT) was conducted, and various quality indicators were measured during this time, such as: pH, titratable acidity, colour, total aerobic count (TAC) and sensory analysis.
The test was performed on smoothie beverages purchased from Romanian market for 24 days, the samples being stored at three different temperatures: 5°C, 10°C and 15°C. The analysis of the quality parameters showed that the pH, titratable acidity and the measurement of colour suffered a minor value modification, while the TAC number increased as the storage temperature increased. The results of the research were analysed using Arrhenius equation and Q10 Rule.
The TAC changes followed an apparent first-order kinetics, their rates and activation energies were estimated from the experimental data (as a plot of temperature vs time).
The fruit smoothies had an estimated shelf life of about 57 days (at 5°C) and 50 days (at 7°C) using the Arrhenius equation and an estimated shelf life about 49 days using the Q10-Rule, after extrapolating the data to real temperature conditions.

Key words*: Accelerated shelf life, Arrhenius, Q10.*

INTRODUCTION

Shelf life may be defined as the time in which a food product is considered safe for consumption. Consumer acceptability of a food product may be a critical parameter to determine its shelf life (Freitas & Costa, 2006).
Shelf life is commonly estimated by two different stability testing procedures: real time stability tests (which are time consuming and expensive if the product has a long-term shelf life) and accelerated stability tests (those can be successfully used for stable products having short and long expected shelf life). Therefore, the products are tested under accelerated conditions to increase the rate of chemical, microbiological and/or physical degradation (Haouet et al., 2018).
Sensory tests are the most important tests to be carried out during a shelf life study to assess the changes in perceived product attributes during storage. The changes in the attributes will relate directly to the stability of the product. If the sensory changes are not caused by microbial growth and spoilage, as for the most part of the cases, the degree of change in sensory characteristics must be directly related to product acceptability.
Both analytical and hedonic sensory tests can be used to gain knowledge about product stability (Kilcast, 2000). In ASLT, a product is stored at elevated stress conditions (such as temperature, humidity, and pH) (Man, 2002). The simplest and most used method of ASLT is based on employing only a single factor to expedite the deterioration process. The simplicity of such a method is related to both the experimental procedure and the extrapolation of data (Steele, 2004).
ASLT is applicable to any deterioration process that has a valid kinetic model. That process may be chemical, physical, biochemical or microbiological. The principles of the ASLT will be the same in all cases. To predict the actual shelf life, one needs to know or to evaluate how the deterioration process behaves as a function of time (Mizrahi, 2004). In order to determine the shelf life of a product, it is important to understand the relationship

between different factors affecting its shelf life (Subramaniam, 2007).
Among many potential accelerating factors, temperature is the most used within ASLT (Fu, 1997).
ASLT using various kinetic models are useful for assessing the effects of temperature changes on product quality (Jedermann et al., 2009).
Each product has a specific mode of spoilage (*i.e.* rancidity, moisture loss/gain, organoleptic changes), so every study is tailored to the specific product (Haouet et al., 2018).
The aim of this study was to compare the shelf life of the smoothie beverage with the estimated results obtained by two different accelerated aging methods. Another objective was to study the influence of the temperature difference (increased in small stages) over the shelf life. The fruit smoothies were tested for about a month at various temperatures (5°C, 10°C and 15°C) and data obtained with ASLT was calculated through reaction order, Arrhenius equation and Q10 Rule.

MATERIALS AND METHODS

All reagents used in the experiments were of analytical grade.
Fruit smoothies generally have a short shelf life (of about 20 days) and are usually preserved by mild pasteurization. The samples tested in this study, were preserved by HPP technology and had a shelf life of 60 days, according to the package. The fruit smoothies can deteriorate by microbiological growth, physical-chemical degradation and sensory changes like: loss of colour and flavour.
The composition of the tested samples was as follows: 40% banana, 31% pineapple juice, 10% orange juice, 7% apple juice, 7% coconut milk, 5% lemon juice. After purchasing the fruit smoothies (from Romanian market) the samples were immediately analysed and then stored under refrigeration conditions (at 5°C) in a refrigerator and thermal stress conditions at 10°C and 15°C (under UV light) in a climatic test cabinet TK 120 (Nuve).
During storage, analyses were performed every six days for 24 days. The parameters affecting the microbiological, chemical and sensory quality of the smoothie were analysed in triplicates.

A. Physical-chemical analyses

Colour measurement (spectrophotometric method)

The colour of a fruit smoothie is an important quality criterion. In some fruit smoothies a relatively light colour is expected for a good quality, such as apple or grape juices. However, in red/black fruit smoothie a deep red/purple colour is expected (Turker, 2004; Rocha, 2003). The colour was determined spectrophotometrically by measuring the absorbance or transmission in the visible region of the spectrum using a UV-VIS (Shimandzu) spectrophotometer.
For products having a yellow/brown colour (apple, pear, white grape juices) the absorbance is measured at 430 nm, while for products having a red/black colour (blackcurrant, raspberry juices) the absorbance is measured at 520 nm, according to IFU Analysis No. 80/2010.

Determination of colour index

To give an indication of the extent of oxidative browning of the anthocyanin pigments in red/black juices, the measurement of the "brown index" is employed. It is determined by the next formula (1) using the ratio between the absorbances of the product at 430 nm and 520 nm (Muche et al., 2018).

$$\textbf{Brown Index}=\frac{A430nm}{A520nm} \quad (1)$$

For products of a red/black colour the "blue index" is also measured, which is the ratio between the absorbances at 580 nm and 520 nm (2). Absorbance was measured after diluting the fruit smoothies with distilled water (1:10).

$$\textbf{Blue Index}=\frac{A580nm}{A520nm} \quad (2)$$

The conductivity and the pH determination

A 50 ml volume of each sample were placed in Berzelius flask. The conductivity and the pH of the smoothie samples were determined using a digital pH/EC/TDS/Temperature Meter-HI 991301 (Hanna Instruments).

Acidity value (AV)

The method is used for the determination of the citric acid in liquid food products by titration with sodium hydroxide 0.1 N. Each 10 ml of

sample was placed in a Berzelius flask and mixed with distilled water until a final volume of 50 ml. The mixture was titrated with NaOH until a pH of 8.3. All determinations were performed in triplicate and the results were expressed as g/L citric acid. The acidity was performed using a potentiometric titrator HI 901 (Hanna Instruments).

Microbiological analyses

The samples stored in refrigeration conditions (at 5°C) and temperature stress conditions (at 10°C and 15°C), were diluted in NaCl 0.9% to obtain serial dilutions of 10^{-1} and 10^{-2}. In this method, a fixed amount of inoculum (1 ml) from the sample is placed in the centre of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 20 mL) is then poured into the Petri dish containing the inoculum and mixed well. Total aerobic count (TAC) was analysed using plate count agar and after incubating the plates at 30°C in a cooled incubator ES120 (Nuve) for 72h according to guidelines from EN ISO 4833. All analyses were performed in triplicate and results were expressed as colony-forming units per millilitre (cfu/ml).

B. Sensory analyses

Difference tests (e.g. paired comparison, duo-trio and triangle tests are designed to determine whether two samples can be distinguished from each other by sensory analyses. Difference tests can be used to determine whether a noticeable change has occurred in a food's appearance, flavour, or texture as a result of storage, of changes in processing methods, or of alteration of an ingredient (Taylor, 2004).

C. Data analyses via kinetics

The Arrhenius method

In general, the chemical reaction rate speeds faster in higher temperatures, meaning that the decrease of product quality occurs rapidly. The shelf life of food products may be determined by the Arrhenius models through extrapolation to real storage conditions (Phimolsiripol & Suppakul, 2016). The Arrhenius equation ((3) and (4)) is a formula for the temperature dependence of reaction rates that has a vast and important application in determining rate of chemical reactions and calculation of the activation energy, which is the best example of such a validated model (Laidler, 1988).

$$\mathbf{K= A}e^{\frac{-Ea}{RT}} \quad \textbf{(3)}$$

Where: K is the reaction rate constant; A is the constant; Ea is the activation energy (kcal/mol); R is the universal gas constant (R = 1.987 cal · mol^{-1} · K^{-1}); T is the absolute temperature (K).

The above formula can be modified as follows:

$$\mathbf{lnK= -}\frac{Ea}{R}x\frac{1}{T}\mathbf{+ lnA} \quad \textbf{(4)}$$

The degradation rate depends on the activation energy for the chemical reaction and it is specific to each product. We don't always have to deal with higher order equations; in many cases, the observed responses of different orders of reactions are indistinguishable for products that degrade slowly (Haouet et al., 2018)

The equation has shown to embrace empirically to a huge range of chemical reactions among those observed in food systems (Decker et al., 2010). This explains why Arrhenius equation is commonly applicable in accelerated shelf life tests regarding food products.

The Q10 Rule

Q_{10} value is a frequently used parameter to describe the temperature dependence of a reaction rate (equation (5)). It can be estimated via the quality changes at increases of 10°C (Fu, 1997). Q_{10} value is calculated as follows:

$$\mathbf{Q_{10}=}\frac{reaction\ rate\ (T+10°C)}{reaction\ rate\ at\ T°C} \quad \textbf{(5)}$$

can be transformed via the Arrhenius equation (6):

$$\mathbf{Q10=}e^{\frac{-Ea}{R}[\frac{10}{T(T+10)}]} \quad \textbf{(6)}$$

Where: E_a is the activation energy (kcal/mol); R is the universal gas constant (R = 1.987 cal/mol) and T is the absolute temperature (K) (Park et al., 2018).

RESULTS AND DISCUSSIONS

Triangle test and the training program for panellists - this is a critical element in achieving the successful operation of the program. Without sensory training the panellist's judgements will be based on their own preferences. The panellists were trained using a reference product to illustrate the target quality, also varying degrees of deviation (Kilcast, 2010). Panellists are provided with three coded samples, one different and two identical, and asked to select the different sample.

Panellists are required to select the different sample even if they cannot discern any differences among the samples.

The panellists received 2 sets of samples:

- In the first set of samples they received two glasses with the fruit smoothie at 5°C and one glass with the fruit smoothie at 10°C (as the different sample).
- In the second set of samples they received two glasses with the fruit smoothie at 5°C and one glass with the fruit smoothie at 15°C (as the different sample).

The two different samples (A and B) are presented to the panellists in sets of three.

Panellists receive either two A's and one B, or two B's and one A. The three samples are presented in identical sample containers coded with 3-digit random numbers. All three code numbers on the samples presented to each panellist must be different, even though two of the samples are identical. The responses of the panellists are presented in Table 1.

Table 1. The number of the correct responses for the triangle test

Day	*n*	α	*X*	*Y*
0	18	0.05	1	2
6	18	0.05	5	6
12	18	0.05	5	8
18	18	0.05	9	10
24	18	0.05	13	15

Where: n - the number of panellists; α - risk level; x - the number of correct responses for the first set of samples; y - the number of correct responses for the second set of samples.

If the number of correct responses is greater than or equal to 10, according to SR EN ISO 4120:2007 (value corresponding to the number of panellists - n, and to the risk level chosen for the test - α), it was concluded that perceptible difference exists between the samples.

Under a null hypothesis the test statistic follows an F distribution to each source of variability, we can associate a p-value (significance level of 5%) that indicates the consensus amongst panellists for the sensory attributes (Lê, 2014).

It was concluded that perceptible difference exists between the samples.

From Table 1 it was concluded that there is a perceptible difference in the last day of the test for the samples stored at 10°C and 15°C.

The changes in colour

The increase of temperature and longer storage periods caused not only reduced anthocyanin concentrations (the blue index decreased) but also other changes including, brown colour development and haze formation. The correlation between the changes of colour and temperatures is presented in Table 2.

The colour of the samples was monitored during storage at 5°C, 10°C and 15°C for 24 days. The brown colour and blue colour development are presented in Figure 1 and Figure 2.

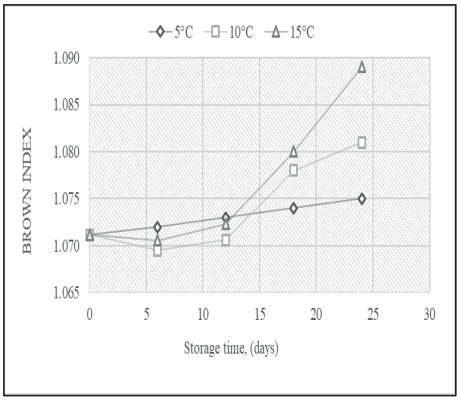

Figure 1. Change of browning index during storage at different temperatures for 24 days

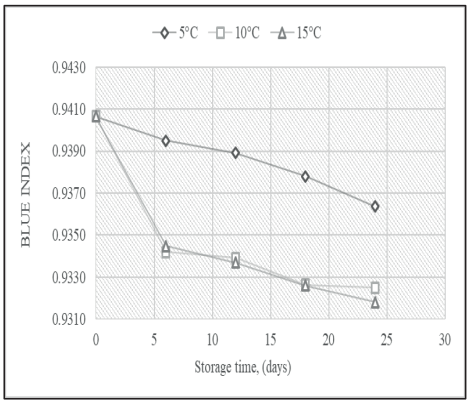

Figure 2. Change of blue index during storage at different temperatures for 24 days

The chemical and microbiological changes

Extrapolation from stressed testing conditions to ambient conditions is usually performed based on established relationships between kinetic parameters and the storage environment.

In general, the rate of chemical reactions will accelerate at higher temperatures, which means the decrease in product quality is faster. TAC and AV are critical quality parameters that affect the changes in fruit smoothies.

The correlation between the chemical and microbiological parameters and temperature is presented in Table 2.

The shelf life is determined based on extrapolation to the storage temperature in the reaction order equation (Nurhayati et al., 2017).

Table 2. Chemical and microbiological properties (pH, conductivity, TAC and AV) of fruit smoothies during storage at different temperatures (5°C, 10°C and 15°C) for 24 days

Temperature	*Day*	*pH*	*AV [g/L]*	*TAC [cfu/ml]*	*Conductivity [mS]*
5°C	***0***	3.95	5.268	<1	>3,999
	6	3.94	5.290	<1	
	12	3.94	5.321	<1	
	18	3.92	5.382	<1	
	24	3.92	5.401	19	
10°C	***0***	3.95	5.268	<1	>3,999
	6	3.93	5.290	<1	
	12	3.91	5.374	10	
	18	3.90	5.391	$17*10^3$	
	24	3.89	5.421	$1.03*10^4$	
15°C	***0***	3.95	5.268	<1	>3,999
	6	3.92	5.301	17	
	12	3.90	5.395	$1.06*10^4$	
	18	3.89	5.505	$2.3*10^5$	
	24	3.87	5.390	$15.91*10^6$	

The zero-order reaction is obtained by plotting the quality changes with time (TAC vs Storage time) and (AV vs Storage time). The first order reaction is obtained by plotting (1/TAC vs Storage time) and (1/AV vs Storage time). The second order reaction is obtained by plotting (ln (TAC) vs Storage time) and (ln (AV) vs Storage time). From these plots we obtain the rate of degradation (k) for the samples stored in stress condition. The selection of reaction order of TAC and AV, in fruit smoothies is done by obtaining a determination coefficient (R^2>0.9) in each linear regression equation. The R^2 values were higher than 0.9 for the zero-order reaction, values presented in Figure 3 and Figure 4.

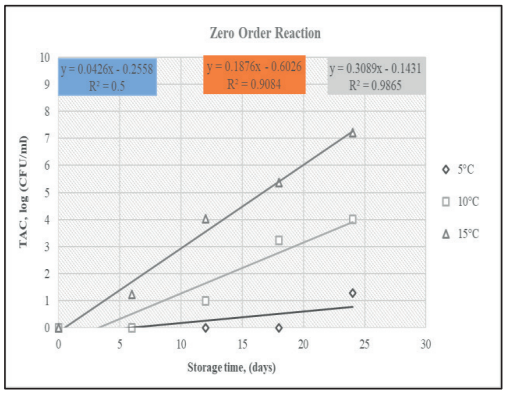

Figure 3. Change of TAC values, during storage at different temperatures

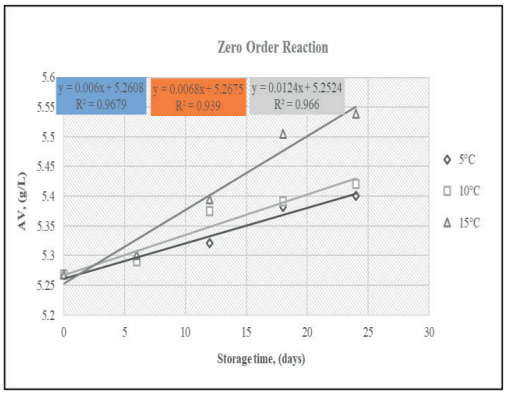

Figure 4. Change of AV values, during storage at different temperatures

The correlation between temperature and rate of degradation can be seen after determining the value of K (rate constant) and E_a (activation energy) in the Arrhenius plot equation for the TAC and AV changes, seen in Figure 5 and Figure 6. For the Arrhenius model we plot the k (degradation rate) from the Zero Order kinetic equation with 1/T (temperature in °Kelvin) obtaining the following linear regression equation:

$$\ln K = \frac{-Ea}{R}\frac{1}{T} - \ln A \qquad (7)$$

where ln A is intercept and E_a/R is the slope (Wahyuni et al., 2018).

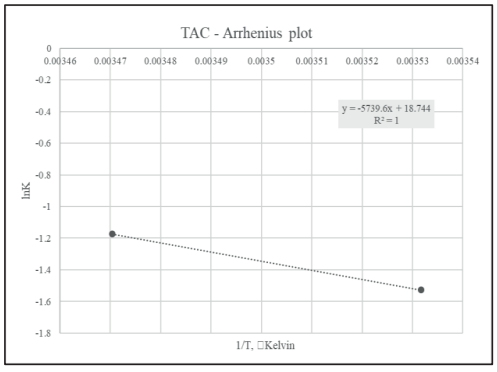

Figure 5. Linear regression curve for Arrhenius plot (TAC)

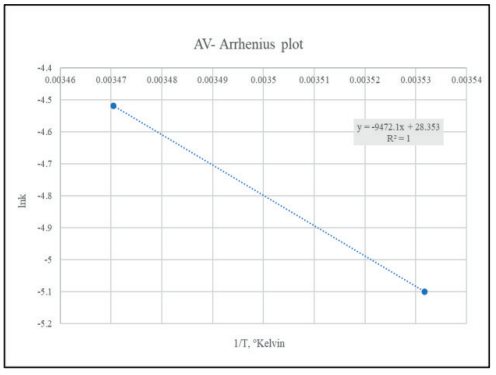

Figure 6. Linear regression curve for Arrhenius plot (AV)

Q10 and E_a were calculated allowing to obtain a predictive evaluation of the product shelf life at the recommended temperature. The parameters for the kinetic model are presented in Table 3.

Table 3. The parameters for the kinetic model

Quality indicator	*Temperature T (°Kelvin)*	*Reaction order*	*Constant K*	*Ea (kcal/mol)*	*Q10*
TAC	278.15	Zero order	0.1509	13.71	2.05
	279.15		0.1615		1.90
	280.15		0.1748		1.77
AV	278.15	Zero order	0.0033	22.63	3.26
	279.15		0.0037		2.89
	280.15		0.0042		2.56

We extrapolated the data to the real temperature conditions and estimated the shelf life by using both Arrhenius equation and Q10 Rule equation (we used only the microbiological changes, because these had a faster evolution than the acidity value). The results are presented in Table 4.

Table 4. Estimated shelf life according to storage temperature using the Arrhenius equation and Q10 Rule equation

Temperature (°C)	*Estimated shelf life (days) using Arrhenius eq*	*Estimated shelf life (days) using Q10 eq*
5°C	57.51	49.12
6°C	53.41	45.62
7°C	49.41	42.62

CONCLUSIONS

After analysing the quality changes associated with increased values of the temperature and estimated the shelf life by using these two methods (based on extrapolation to storage temperature), we obtained a minimum shelf life of the fruit smoothies of 49 days at 5°C for the Q10 Rule and 58 days for the Arrhenius equation. If the temperature increases by 1°C the estimated shelf life of fruit smoothie decreases by 3-4 days and if the temperature increases by 2°C the estimated shelf life of fruit smoothie decreases by 7-8 days.

From the data obtained we observed that the Q10 Rule equation can provide us the minimum durability of the product and that the Arrhenius equation can provide us the maximum shelf life.

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