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STUDY ON ACCELERATED SHELF-LIFE TESTING OF UHT COW MILK

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ABSTRACT

Real time shelf-life determination of UHT milk can be very time consuming and expensive. New procedures must be developed to predict or estimate shelf-lifes of a food product without running a full length storage trial. The aim of this study was to gain a deeper know-ledge concerning the general and accepted way of performing accelerated storage tests on diary product with oxidation as the delimiting quality failure parameter. The best oxidative indicator was the total hexanal content. The measurement method of hexanal by using GAS Chromatography (GC) together with Q10 predictive method was the procedure for indirect determination of shelf-life of UHT milk. The shelf-life was estimated to be 217 days when is styored at 25 ^{o}C, 81 days at 35 ^{o}C and 35 days at 45 ^{o}C.

INTRODUCTION

Ultra high-temperature (UHT) milk is milk processed at a combination of times and temperatures in the ranges of 130-150 ^{o}C, for 2-8 seconds and aspetically packaged to produce a commercially sterile product. The product requires no refrigeration and has a longer self-life.

Shelf-life is defined as the time during which the food product will:

- remain safe;
- be certain to retain desired sensory, chemical, physical and microbiological characteristics;
- comply with any label declaration of nutritional data, when stored under the recommended conditions.

A dairy food is inherently perishable and depending on its physical and chemical properties, microbiological quality and the storage conditions, there will come a point when either its quality will be unacceptable or it will become harmful to the consumer. At this point it has reached the end of its shelf-life. The ability to predict this is of great value to the dairy industry when defining storage and distribution conditions and limits, formulating products, assessing manufacturing processes and doing quantitative risk assessement. Many food-products have some variation of open shelf-life dating marked on their containers. These dates help the consumer to decide haw long the product may be stored prior consumption and help with stock rotation in grocery stores. Therefor food manufacturer conduct studies to determine the shelf-life of their product. Two methods are used: direct and indirect methods. The direct method involves storing the product under preselected conditions for a period of time (longer than the expected shelf-life), and checking the product at regular intervals to see when it begins to spoil.

The indirect method uses accelerated shelf-life testing (ASLT) or predictive modeling.

Accelerated shelf-life testing (ASLT) offers a way to estimate shelf-life without having to wait a long time for the answer.

Shelf-life studies and ASLT require a profund knowledge of the constituents of the food, the process, the microbiological safety factors, the main modes of quality determination and the intended storage condition (Mizrahi, 2004). The shelf-life of a

product depends on four main considerations: formulation, processing packaging and storage conditions. For packaging and store conditions, the keeping properties of the product are function of its microenvironment. This include temperature, relative humidity, gas composition, light and pressure. Any or all of these components may be manipulated to lengthen shelf-time.

The concept of accelerated studies is to increase reaction causing factors in order to accelerate changes in the physical, chemical or microbiological characteristics of food products. Increasing storage temperature is the most popular method used. By subjecting the food to such a controlled environment the deterioration rate will ne increased, resulting in a shorter time to product failure.

Ultra high temperature (UHT) processed milk has a reported shelf-life of 6 to 9 months at room temperature (25 ^{o}C). The sensory quality and the shelf-life of UHT milk is governed by the progression of various physic-chemical and biochemical changes after processing. The main changes are due to proteolytic, lipolytic, oxidative and Maillard type reactions (Datta and Decth, 2003). Although UHT processing inactivates most bacteria, some heat-stable enzymes of native and bacterial origin can survive and cause shelf-life limiting defects. Proteolysis is associated with release of tyrosine thet contribute to the development of off-flavours. The B-lactoglobulin-K-casein complexes during heat treatment increase the viscosity of milk (Datta and Decth, 2003).

Lypases can hydrolyse triacylglycerols with the release of medium and short chain fatty acids. Rancidity and off flavours can occur if triglyceride contains volatile, short fatty acids mainly butyric acid (C4:O) and lauric acid (C12:O) as in dairy fats.

The same hydrolytic rancidity mechanism can be a result when triglycerides are exposed to water. Water hydrolyses and split the ester bonds, breaking the structure of triglycerides into mono or diglycerides and free fatty acids (FFA). This reaction is catalyzed by either high temperature or due to the enzyme lipase or a combination of bouth (Sewald and DeVries, 2003). The amount of FFA can be measured but in not a true measure of the reaction rate.

The oxidative rancidity reaction is a spontaneous reaction of unsaturated fatty acids with atmospferic oxygen (Coultate, 2009). The reaction can be accelerated at higher temperatures (Shahidi and Wiley, 2005). The reaction requires some initial activation energy in order to remove a hydrogen atom from unsaturated fatty acid (UFA). Higher temperatures and the presence of double bonds enabled this (Barriuso et al, 2013).

Also oxygen reacts with a double bound on an UFA resulting hidroperoxides and radicals. Further oxidation steps lead to the breakdown of hydroperoxides and production of carbonyl compounds such as aldehydes. Identification of these secondary oxidation products has a great significance. The perception of rancidity is often due to this amount of volatile aldehides such as propanal, pentanal, hexanal, heptanal and nonanal (Barriuso, 2013). Hexanal is the most important volatile degradation compound, deriving from chemical oxidative rancidity mechanism, and is created from 13-hydroperoxidisomer derivative (Coultate, 2009). If free oxygen attacks for example carbon number 13 on the fatty acid molecule it results in the formation of a 13 hidroperoxides, Linoleic acid (C18:2) gives rise to the formation of 9-13-hidroperoxidis.

Hexanal is more frequently measured as it formation is higher than of other secondary oxidation products (Barriuso et al, 2013).

The aim of this study was to gain a deeper knowledge concerning the general and accepted way of performic accelerated storage test on dairy product with oxidation as the delimiting quality failure parameter.

MATERIALS AND METHODS

Experimental design

The concentration and quantity of n-hexanol in low fat UHT milk can be detected in 10 minutes by the GasCromatography/MS (GC/MS) analysis.

The sample must be kept cold to minimize the risk for oxidation before analysis. Acording to Sewald and DeVries, 2003, a homogenous portion of the sample must be collected, and at least six samples should be analized at the test temperature within a range of 20-60 ^{o}C. The formation of primary and secondary oxidation products were observed between 25 and 75 ^{o}C, according to the literature. Common test temperature are 10 ^{o}C apart (e.g. 20, 30, 40 and 50 ^{o}C). It is preferable a control sample, stored to 0 ^{o}C. Analysis each weck is commonly performed and each testing temperature should have at least six data points over time to make the study results statistically reliable. At least two temperature are required, but three or four is an ideal number for more precise predictions of shelf-life (Hough et al, 2006). A test temperature over 50 ^{o}C in not good, n-hexanal can be converted to hexanoic acid or to n-hexanol at higher pH by alcohol dehydrogenase with small impact on sensory attributes in food products (Mellor et al., 2010).

According to the literature a shelf-life study concerning oxidative stability could be carried out for 60 days to generate good test results.

Sample preparation and analysis method

For this study milk from three different batches (3 replicates) of low fat UHT milk was collected on the day of production and stored at 25, 35 and 45 ^{o}C for 210 days. The gross composition (protein, lactose and fat content), microbiological quality (*Enterobacteriaceae* level) and volatile aldehyde compounds were analysed by the Sanitary Veterinary and Food Safety Laboratoriers Timisoara and Dolj.

The analysis of variance was performed to identify differences in gross composition of milk and microbiological quality and also to identify any changes over time (0, 30, 60, 120, 150, 180 and 210 days). Volatile aldehyde components were extracted by solid phase micro extraction (SPME) and were analysed by GC/MS.

A homogenous portion of the sample is suspended and mixed with boilig water containing an internal standard (4-heptanone). The sample is then heated for a specified amount of time. Then a sample of the headspace is taken and injected into GC analyzer. The hexanal released is detected and quantified via comparasion of the internal standard.

Kinetic mathematical models

Shelf-life prediction is based on reaction kitecics where the acceleration of the deterioration rate is direct time and temperature depended. The quality loss follows the equation $dQ/dt = K(Q_A)^n$, where dQ/dt is the change in the measurable qualiry factor A, with time, K is the rate constant in appropriate units, and n is the order of the chemical reaction of the quality factor. The order of reaction for most quality attributes in food products is either zero, first or second. Rancidity reactions are first under reaction since it is degradation reactions. The rate of quality decrease is exponential. Plotting the log scale values versus time, makes the experimental data easily interpretable.

The rate constant (K) from the liniar relationship can be determined:

$K = K_0 e^{\left(\frac{-E}{R}\right)}$; $\ln K = \ln K_0 - \frac{E}{R}\left(\frac{1}{T}\right)$, where:

K = a reaction rate constant to be estimated;
K0 = a constant independent of temperature
EA = activation energy (Kcal/mol) specific for a certain reaction;
R = is the molar gas constant 8.31 J/K/mol;
T = temperature (in ^{o}Kelvin).

Once the K value, the slope, is determined, the activation energy (EA) can be determined using Arrhenius equation.

Arrhenius equation

A chemical deterioration process require a certain amount of energy to get started and this is called the activation energy (EA) given in Kcal/mol. If the temperature gets higher, the acceleration of the reaction will increase. The reaction rate constant K quantifies the speed of the reactive. In a first order reaction, EA should result in a plot of the lnK and the reciprocal of temperature, 1/T, generating a slope. The data can be extrapolated further in order to find out the oxidative rate for a desirable temperature or to find the accelerating factor.

Q10-modeling

Q10 is the increase in the rate of the reaction when the temperature is increased by 10 ^{o}C.

The Q10- value of 2 is usually found for enzymatically induced changes or degradation of pigments and flavors. The Q10- value can also be calculated from the ratio between the rate constant at KT and KT+10. This means in practice that the measurements (in mg of hexanal per 1000 g sample) can be used directly.

$$Q_{10} = \frac{K\ +1}{K} = \frac{S}{S\ +1\ ^{\circ}C}$$

KT + KT + 10 = recation rate constants at temperature T + T + 10 ^{o}C;
T and T + 10 = temperatures;
ST + ST + 10 = related shelf-life estimates at temperature T and T + 10.

Accelerating factor

Accelerated shelf-life tests are needed to find a proper acceleration factor (AF) for the tested product.

Arrhenius equation and Q10- modeling method are mainly two possible procedures. The EA is the information needed to determine the AF. The AF enables the conversion from accelerated temperature is back again to actual storage condition at normal temperatures.

$$AF = \exp\left[\frac{E}{R}\left(\frac{1}{T} - \frac{1}{T}\right)\right]$$

AF = accelerating factor;
exp (x) = e^{x};
Tu = actual storage temperature;
Ttest = accelerated test temperature.

RESULTS AND DISCUSSION

The freshly packed low fat UHT milk used showed satisfactory milk composition and microbiological quality (Table 1).

Table 1

Milk composition and microbiological quality of freshly packed UHT milk

	Batch 1	Batch 2	Batch 3
Milk composition			
% fat (g/100g)	1,80 ± 0,02	1,81 ± 0,02	1,82 ± 0,02
% protein (g/100g)	3,48 ± 0,02	3,50 ± 0,02	3,49 ± 0,02
% lactose (g/100g)	4,95 ± 0,01	4,91 ± 0,02	4,90 ± 0,01
Microbiological quality:			
Enterobacteriaceae CFU/ml	< 1	< 1	< 1

The mean content of fat, protein and lactose was 1,81 ± 0,01, 3,49 ± 0,01 and 4,92 ± 0,02% (Bath 1 + Bath 2 + Bath 3).

The microbiological quality of the UHT milk was very high with counts of < 1 CFU/ml. The microbiological quality was maintained trroughout the duration of the study at ambient temperature (Table 2).

Table 2

The effect of storage time on microbiological quality of UHT milk

Enterobacteriaceae							
Time (days)	0	30	60	120	150	180	210
Batch 1 (CFU/ml)	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Batch 2 (CFU/ml)	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Batch 3 (CFU/ml)	< 1	< 1	< 1	< 1	< 1	< 1	< 1

Hexanal content showed the most change over the time period. Regression curves and R-squared:

at 25 ^{o}C: $Y = 0,07x + 13,26$
$R^2 = 0,876$
at 35 ^{o}C: $Y = 0,27x + 13,93$
$R^2 = 0,912$
at 45 ^{o}C: $Y = 0,64x + 49,15$
$R^2 = 0,840$

Hexanal content is suitable for estimating the shelf-life parameters. Hexanal parameters for UHT milk are presented in Table 3.

Table 3

Hexanal parameters

Temperature (^{o}C)	Rate constant K Hexanal score/day	Coefficient of determination (R^2)	Acceleration factor	
			25 – 35 ^{o}C	35 – 45 ^{o}C
25 ^{o}C	0,1380	0,876	3.97	2.57
35 ^{o}C	0,3714	0,912		
45 ^{o}C	0,8666	0,840		

Shelf-life of UHT milk stored at 35 ^{o}C and 25 ^{o}C was estimated at 81 days and 217 days, respectively.

The acceleration factor was determined to be 3,97 and 2,57 when the storage temperatures increased from 25 ^{o}C to 35 ^{o}C and from 35 ^{o}C to 45 ^{o}C, respectively. The rate of oxidative deterioration of the milk at 35 ^{o}C will be more than 3,9 times faster, and the rate at 45 ^{o}C will be 10,2 times faster, than rate at 25 ^{o}C. This means that, for on estimated shelf-life of 217 days, future hexanal in ASLT have to be conducted for 81 days at 35 ^{o}C or only 35 days at 45 ^{o}C.

CONCLUSIONS

The hexanal content in accelerated shelf-life test was successfully applied to low fat UHT milk. The shelf-life of the designated low fat UHT milk was estimated to be 217 days when is stored at 25 ^{o}C.

Higher temperatures of storage negatively affected the shelf-life of UHT milk. Shelf-life was shortered to 81 days and 35 days, when is stored at accelerated temperature of 35 ^{o}C and 45 ^{o}C, respectively.

An accelerated shelf-life study should always be performed in parallel with a full-length study and with sensory evaluation program. The sensory evaluation program is considered as major limiting parameter, due to the consumers acceptance.

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