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Testing Shelf-life of an Immune-enhancing Power Bar for Immune-deficient Patients

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ABSTRACT

An n-order mathematic expression was used to evaluate the shelf-life quality and deterioration of a newly developed power bar for immune-deficient patients. Accelerated shelf-life testing conducted at 40, 50 and 60°C was used to predict the shelf-life of the product at usual storage conditions. The Arrhenius model that was used indicated that the power bar can be stored for 168 days at 4 ± 1°C, 105 days at 9 ± 3°C, 56 days at 15 ± 5°C, 21 days at 30 ± 2°C, and only 6 days at 42 ± 3°C. The calculated Q_{10} values were found to be in the range of 1.5-2.0, which is in the range for lipid oxidation in various food products reported in the literature.

Keywords: food, HIV, *n*-hexanal, quality, storage, use by date

INTRODUCTION

Inadequate food intake is the first significant cause of death in case of Human Immune Virus (HIV)/Acquired Immuno-Deficiency Syndrome (AIDS) affected peoples (World Health Organization 2003; Zello 2006). Nutritional and micronutrient deficiencies play an important additive role in immune degradation (Anabwani and Navario 2005). In the case of peoples living with HIV/AIDS, there is a need to boost their immune systems by means of nutrition (WHO 2003), specifically in the case of highly active anti-retroviral therapy (Hogg *et al.* 1999). Research on boosting the immune system of peoples living with HIV/AIDS by means of nutrition has been reported and mainly focused on the production process of energy bars, food multimixes, and porridges for immune-deficient patients (Meyers *et al.* 1995; Cassano and Drioli 2004). In order to contribute to solve this problem, a power bar, for immune-deficient children was developed at the Vaal University of Technology. This is a powerful immune-enhancing meal formula, combining a comprehensive blend of nutrients in a pleasant-tasting, precooked, ready-to-use, nutritious and ethnically acceptable food. In theory it is acceptable to mix ingredients from different sources (carbohydrates, proteins, lipids) and fortify these by adding vitamins and some essential minerals (selenium, iron, zinc, calcium) to meet energy, protein and micronutrients needs (Amuna *et al.* 2004; Cassano *et al.* 2004). The effects of time and storage conditions in the nutrient contents and shelf-life of this power bar, however, are not yet well understood. As the storage and environmental conditions may affect its nutritious quality, the determination of the shelf-life of this new food product is required. The research on which this article is based thus aims at determining the shelf-life of the power bar specifically the *n*-hexanal production during storage at various temperatures. The focus of this research is only on one of the physicochemical attributes of shelf-life, which consists of the determination of oxidation rate.

MATERIALS AND METHODS

Product development

The main ingredients used to prepare the power bar were margarine, brown sugar, orange juice, cake flour, soy flour, soy milk powder, baking powder, and a variety of toppings. The power bars were prepared as follows: An oven was preheated to 160°C, then the margarine and brown sugar were placed in a saucepan, and heated gently until melted. The mixture was removed from the heat and the orange juice was added. Following that, the cake flour, soy flour, soy milk powder and baking powder were sifted together and the melted margarine mixture was added. The mixture was then pressed in a lamington pan of 20 × 30 centimetres (cm) and baked for 15 minutes at 160°C.

Shelf-life testing

Components of shelf-life testing included assessing the physico-chemical and microbiological attributes of the prepared energy bars. The physico-chemical attributes consisted of the determination of oxidation rate. This experiment was conducted at three accelerated shelf-life test temperatures and this information was used to model the shelf-life of power bars at ambient conditions. The Arrhenius model (Labuza and Riboh 1982), supplemented by the linear model (Robertson 1993), was used to predict the shelf-life of power bars at ambient conditions.

Storage conditions

For storage under various conditions, power bar samples, weighing 100 grams (g) each, were placed in wide-mouth 473 millilitre (ml) mason jars (Ball, Alltrista Corp., Muncie, IN). The storage temperatures were 40, 50 and 60°C, respectively, and the water activity (a_w) values of the samples were adjusted to the range of 0.29-0.38 using moisture-absorbent sachets (silica gel pillow pack, Desiccare Inc., Santa Fe Springs, CA). Temperatures and relative humidity of the chambers were monitored using a data logger (model TL 120, Dickson Company, Addison, IL) as described by Lee *et al.* (2002).

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Determination of *n*-hexanal

Previous studies have shown that the *n*-hexanal level correlates well with the rancid attribute by comparing sensory and instrumental methods (Lee *et al.* 2002). The hexanal level was also found to be a good indicator of oxidative rancidity in many other food systems (Bovell-Benjamin *et al.* 1999). We used the *n*-hexanal variation to predict the shelf-life of the energy bars. *n*-Hexanal was determined as the 2,4-dinitrophenylhydrazone derivative with high performance liquid chromatography (HPLC), according to the method of Matoba *et al.* (1985), except for the use of perchloric acid instead of phosphoric acid. Ten g of energy bar samples were ground in a mortar with liquid nitrogen, and then homogenised at room temperature in 0.5 ml of perchloric acid with a homogeniser of the Potter-Elvejhem type. The homogenate was used for determining *n*-hexanal.

The analyses were performed using the Perkin Elmer HPLC set (Norwalk, USA) comprising of a LC 200 pump, a LC 200 autosampler, LC Column Oven 101 thermostat and LC 235C diode-array detector attached to a Perkin Elmer Turbochrom Chromatography Workstation version 4.1. The Pecosphere C18 150 mm × 4.6 mm, 5 µm particle size reverse phase column (Perkin Elmer, Norwalk, USA) with a column guard (4 × 200 mm; GL Sciences) was used at the flow rate of 1.0 ml min^{-1} and column pressure 6.5 MPa (950 psi). The injection volume was 100 µl and the derivative was eluted with acetonitrile/water/tetrahydrofuran (75: 24: 1, v/v/v) at a flow rate of 1.0 ml/min and detected at 350 nm.

Data analysis

Fifteen samples were prepared and each experiment was carried out in triplicate. Statistical analysis of items was performed by using Excel 2007 and Splus 2000 for Windows 2001.

RESULTS AND DISCUSSION

Theoretical approach to estimate initiation periods of power bar samples

The Arrhenius relationship (Labuza 1982a) or the linear model (Robertson 1993) can be used for describing how much faster or slower a reaction will go if the sample is held at other temperatures (i.e. effect of temperature on *k*). These models were used in this study to extrapolate energy bar shelf-life results from accelerated tests at higher storage temperatures. The aim was to estimate power bar shelf-life results under ambient storage conditions. In the literature, most food-quality deterioration was found to fit either a zero- or a first-order mathematical expression (Labuza 1982a): $-dA/dt = k(A)^n$ where A = a quality attribute measured in some units, n = the reaction order, and k = the rate constant. For either zero- or first-order deterioration, Robertson (1993) also showed that $k_1 t_{S1} = k_2 t_{S2}$ where k_1= rate constant at T_1, k_2= rate constant at T_2, t_{S1} = shelf life at T_1, and t_{S2} = shelf life at T_2.

Application to the determination of initiation and propagation periods of energy bar

The rate of oxidation for the power bar was determined by plotting the *n*-hexanal level versus (vs) storage time. This is in accordance with the fact that the *n*-hexanal level correlated well with the rancid attribute as demonstrated by Lee *et al.* (2002). Hexanal levels also proved to be a good indicator of oxidative rancidity in many other food systems (Bovell-Benjamin *et al.* 1999). From the *n*-hexanal level vs. time plots, linear regressions are derived together with the data points of the initiation period and the propagation period. The *x*-value of the intercept of these linear regressions was determined to be the estimated end of the initiation period. This method of analysis was performed for all samples at each temperature. **Fig. 1** shows *n*-hexanal level vs time plots for energy bars stored at 40, 50 and 60°C respectively.

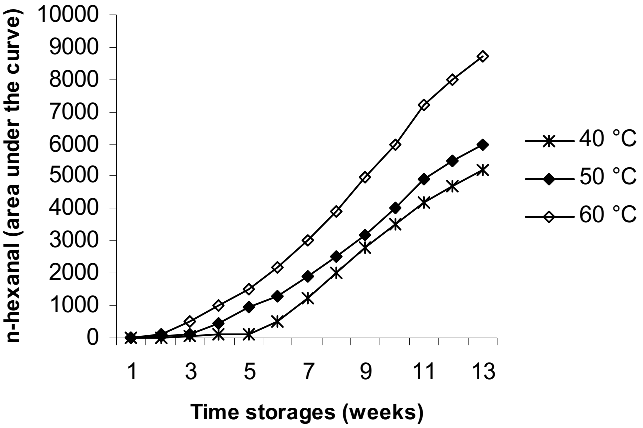

Fig. 1 The extent of oxidation of energy bars stored at 40, 50 and 60°C.

The estimated initiation period at 40°C was found to be five weeks (**Fig. 1**). The same method was used to estimate the initiation period at 50 and 60°C. Initiation periods of three weeks for samples stored at 50°C and one week for samples stores at 60°C were recorded (**Fig. 1**). Because the rate of oxidative rancidity accelerates at the onset of the propagation period, the estimated initiation period could be regarded as a conservative shelf life (*t*s) at the three accelerated storage temperatures. **Table 1** shows regression equations and R^2 values for the initiation and propagation periods together with corresponding estimated initiation periods (*t*s) in weeks for all samples at storage temperatures of 40, 50 and 60°C, respectively.

Shelf-life prediction of the power bar

The general mathematical expression for the Arrhenius relationship is as follows (Labuza 1982c): $k = k_0 e^{-EA/RT}$ (1), where k = rate constant for deteriorative reaction at temperature T, k_0 = constant, independent of temperature (also known as the Arrhenius, pre-exponential collision or frequency factor), E_A = activation energy (J/mole), R = ideal gas constant (8.314 KJ/ mol), and $T°$ = absolute temperature (°K). From the above-mentioned equation 1 and considering Robertson's (1993) zero- or first-order deterioration equation $k_1 t_{S1} = k_2 t_{S2}$, we can derive the following equation: $\log (t_{S1}/t_{S2}) = E_A/2.3R\ (1/T_1 - 1/T_2)$ where k_1 = rate constant at T_1, k_2 = rate constant at T_2, t_{S1} = shelf life at T_1, and t_{S2} = shelf life at T_2. The plot of eq. 3 was made by converting the estimated initiation period (*t*s) at each of the three storage temperatures to log(*t*s) and the storage temperature to 1/(absolute temperature of the storage temperature, *T*), and plotting log(*t*s) vs. 1/*T*. From this plot, Arrhenius shelf-life equations were determined using regression analysis.

The shelf life of the power bar was predicted for respectively:

Table 1 Regression equations and R^2 values for initiation and propagation periods of lipid oxidation and the estimated initiation periods for power bar samples stored at 40, 50 and 60°C.

Storage temperature (°C)	Regression equation and R^2 for initiation period	Regression equation and R^2 for propagation period	Estimated initiation period (*ts*)
40	y = 32.5*x* - 5; R^2=0.87	y = 684.1*x* - 2797.1; R^2=0.84	5 weeks
50	y = 60*x* - 63.33; R^2=0.98	y = 646.8*x* - 1780.4; R^2=0.99	3 weeks
60	y = 100*x*; R^2=1	y = 870*x* - 1817.3; R^2=0.98	1 week

Table 2 Arrhenius shelf-life equation, R^2, and the estimated shelf life (ts)

Storage temperature (°C)	Arrhenius equation	R^2	Estimated shelf life (days)
4 ± 1 (average temperature in the laboratory refrigerator used for the experiment)	$y = 1958.5x - 5.40$	0.998	168
9 ± 3 (average ambient temperature recorded in a wooden cupboard in the Vaal region South Africa in winter during the experiment period)	$y = 1837.2x - 3.85$	0.999	105
15 ± 5 (average temperature in a wooden cupboard in the Vaal region in winter during the experiment period)	$y = 1792.9x - 4.69$	1	56
30 ± 2 (average ambient temperature in the Vaal region in summer during the experiment period)	$y = 1531.2x - 4.67$	0.996	21
42 ± 3 (average temperature in a wooden cupboard in the Vaal region in summer during the experiment period)	$y = 1553.3x - 4.31$	0.997	6

- 4 ± 1°C (average temperature in the laboratory refrigerator used for the experiment);
- 9 ± 3°C (average ambient temperature recorded in the Vaal region South Africa, in winter during the experiment period);
- 15 ± 5°C (average temperature in a wooden cupboard in the Vaal region in winter during the experiment period);
- 30 ± 2°C (average ambient temperature in the Vaal region in summer during the experiment period); and
- 42 ± 3°C (average temperature in a wooden cupboard in the Vaal region in summer during the experiment period).

The results presented in **Table 2** indicate that power bars can be stored for 168 days at 4 ± 1°C, 105 days at 9 ± 3°C, 56 days at 15 ± 5°C, 21 days at 30 ± 2°C, and only 6 days at 42 ± 3°C.

Q_{10} factors: The rate of reactions

With shelf-life data at two temperatures 10°C apart, the Q_{10} factor can be calculated. The Q_{10} factor is defined as the rate of reaction at temperature (T + 10) divided by the rate of reaction at temperature (T), which is simply the inverse of the ratio of shelf life at two temperatures. Q_{10} was calculated using the linear model shelf-life plot as described by Robertson (1993) as follows: $Q_{10} = e^{10b}$, where b is a constant characteristic of the reaction equal to 2.3X (slope of the linear model plot). The calculated Q_{10} values were found to be in the range of 1.5 to 2.0, which was reported to be the range for lipid oxidation in various food products (Labuza 1982a, 1982b).

Predicted shelf-life (indicated by the level of *n*-hexanal) for samples stored in the laboratory refrigerator and samples stored at ambient temperature in the Vaal region in South Africa during winter may be delayed compared to that of the samples stored in a wooden cupboard in the Vaal region in winter (shown by a 2-fold increase in the shelf-life) and samples stored at ambient temperatures and in a wooden cupboard in the Vaal region during summer (shown by a 4- and 16-fold increase in the shelf-life, respectively). However, the recorded average ambient temperature recorded in a wooden cupboard in the Vaal region of South Africa in winter (9 ± 3°C) is not statistically different ($P>0.05$) from the average temperature recorded in a wooden cupboard in the Vaal region in winter during the experiment period but the estimated shelf life is quite different (**Table 2**). The results may have been influenced by the differences in a_w among the samples as the moisture content was found to be different during the experiments.

The samples stored at ambient temperatures were found to lose more water than those stored in the cupboard despite the fact that moisture-absorbent sachets were used to adjust the a_w levels of the samples at the beginning of the experiment. However, the final a_w for different temperatures were found to vary in the range of 0.25 to 0.38. Water was reported to act as a pro-oxidant at very low and very high water activities, and to act as an antioxidant between these two extremes (Labuza 1971). The studied samples may have exhibited differences in the rate of oxidation due to the effect of a_w differences, consistent with results shown in the study by Evranuz (1993).

CONCLUSIONS

A power bar, for immune-deficient patients was developed at the Vaal University of Technology. As part of this product development, its shelf-life at various temperatures was screened. The results from accelerated shelf-life testing conducted at higher temperatures were extrapolated to predict the shelf-life at normal storage conditions. The results indicated that energy bars may be stored for 168 days at 4 ± 1°C, 105 days at 9 ± 3°C, 56 days at 15 ± 5°C, 21 days at 30 ± 2°C, and only 6 days at 42 ± 3°C. This research was conducted on small samples and did not consider other factors like packaging, microbial en enzymatic post-deterioration, which can influence both nutritional and hygienic qualities of the newly developed power bar. There is thus a need for more research to validate this very first data on the power bar for immune-deficient patients.

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