Effect of blanching on kinetic parameters for quality attributes in frozen vegetables

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Abstract: The effects of blanching in three different solutions, tap water as a control, 0.1% carbonate magnesium $MgCO_3$ and 0.2% bicarbonate ammonium NH_2HCO_3 on vitamin C, chlorophyll (a and b), residual lipoxygenase (LOX), polyphenoloxidase (PPO) and peroxidase (POD) activities, color change, organolyptic measurements and quality changes in green bean and Egyptian mallow were studied at zero, 60, 120, 180 days during frozen storage at -12 and -18ºC. No regeneration of LOX and POD activities were detected in frozen-stored samples. The degradations of vitamin C and chlorophylls followed first-order kinetics. The half-time of vitamin C, in blanched green bean ranged from 3.24 to 6.66 months during storage, chlorophylls a and b derivatives ranged from 3.11 to 64.21 months. Blanching of green bean at 96ºC for 2.5 min and 98ºC for 1.5 min increased the half-time of vitamin C, while it decreased those of chlorophylls a and b. Overall results suggested that blanching by 0.2% NH_2HCO_3 was the best treatment to retain quality parameters such as vitamin C and chlorophyll pigments during a storage period of 6 months at -18ºC.

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Key words: Green bean, Egyptian mallow; Shelf-life; Frozen vegetables; Vitamin C; Oxidative enzymes; Color; sensory evaluation.

1. Introduction

Vegetables, an important component of a balanced human diet, are low in fat and energy with high carbohydrate and fibre contents, providing significant levels of some micronutrients. Fresh vegetables have a short durability, and are exposed to conditions that destroy their superior quality in a short period of time before cooking and consumption (**Jabbar et al., 2014**). Seasonality and perishability of vegetables explain the necessity of applying preservation technologies such as freezing to combine shelf life extension with maintenance of sensory and nutrient characteristics. The main factors affecting the final quality of frozen vegetables are: raw material, processing, including blanching treatment method of freezing, post-processing distribution, storage and home-handling **(Labuza and Fu, 1997).** Post-processing temperature conditions and temperature fluctuations determine the rate of quality degradation and the shelf life of frozen vegetables. Improper frozen storage causes evident changes in sensory characteristics that can influence consumer acceptability, but also leads to reduction of nutritive value, mainly in vitamin C, colour and enzymes activities. **(Giannakourou and Taoukis, 2003)**

A considerable body of work on the different modes of quality degradation of different frozen vegetables has been published and reviewed in the earlier and recent literature **(Hung and Thomson, 1989; Pilar Cano, 1999 and Martins et al., 2005).** The current effort is to develop and apply a systematic kinetic and modelling approach to the main quality indices of each product. By establishing the appropriate quality function that describes the time-temperature dependence of the selected mode of degradation, a quantitative tool for shelf life estimation and management is obtained **(Giannakourou et al., 2001).**

Vegetables are a major source of ascorbic acid, a nutrient that besides its vitamin action is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported, such as inhibition of formation of cancer-causing N-nitroso compounds in the stomach **(Hussein et al., 2000)**. The level of vitamin C, besides being an indicator of nutrient value, can be used, in the case of frozen vegetables, as a reliable and representative index for estimating the quality deterioration at any point of the marketing route of a product to its final destination, the consumer. **Kmiecik and Lisiewska (1999)** reported about vitamin C contents of several frozen green vegetables**,** and the effect of pre-treatment and storage temperatures on the preservation of ascorbic acid. They included the nutritional content at steady, low temperature, indicative of frozen practice (*e.g* at -20 and -30ºC), at the beginning, middle and at the end of their commercial shelf-life, without assuming a full, systematic kinetic approach.

Chlorophyll retention has been used as a measure of quality in green vegetables**.** It is well-known that the excessive heating of food products causes

considerable losses in the organoleptic quality of food. Blanching inactivates chlorophylls and enzymes responsible for senescence and rapid loss of green colour. However, chlorophyll degradation is initiated by damaged tissue during blanching and other processing steps **(Tijkens et al., 2001).** Chlorophylls are susceptible to many chemical or enzymatic degradation reactions. The simultaneous actions of enzymes, weak acids, oxygen, light and heat can lead to the formation of a large number of degradation products. **(Koca et al., 2006)**.

Blanching is a thermal process designed to inactivate the enzymes responsible for off-flavours generation. Apart from enzyme inactivation, blanching of vegetables prior to freezing has several advantages, but also a number of disadvantages. The advantages include stabilization of texture, flavour and nutritional quality, destruction of microorganisms and wilting of leafy vegetables, which assists in packaging**.** Process optimization involves measuring the rate of enzyme destruction, so the blanch time is just long enough to destroy the indicator enzyme **(Cano, 1996)**. Many processors utilize a heat treatment sufficient to inactivate peroxidase, one of the more stable enzymes present, and not incidentally, one of the enzymes whose activity is relatively easy to measure. Some researchers suggested that targeting peroxidase leads to a more severe heat treatment than is required for many vegetables and that the enzymes responsible for quality loss, which have been identified, have a lower stability than peroxidase **(Reid, 1998)**. Recently, use of LOX as an indicator of proper blanching has been recommended as more significant in determining storage stability in frozen vegetables **(Bahceci et al., 2005).**

The objective of this work is the development of kinetic models of the nutritional quality (vitamin C, Chlorophylls and enzymes) losses of two widely consumed frozen green vegetables over the entire temperature range of practical interest. These kinetic equations are validated in variable conditions, in order to be used for the reliable prediction of the remaining shelf life of the product at any point of their route to the consumer based on nutritional criteria and the full temperature history of the product.

2. Materials and Methods

Materials:

Green beans *(Phaseolus vulgaris, L.)* and Egyptian Mallow (*Corchorus olitorius, Jew's mallow, Molokhia*) was obtained from a private farm in Ayatte at Giza, Egypt, during 2014-2015 seasons.

Preparation of samples:

Green beans was firstly sorted, washed and it was blanched in three different solutions, tap water as a control, 0.1% carbonate magnesium $MgCO_3$ and 0.2% bicarbonate ammonium NH_2HCO_3 for 2.5 min at 96 ± 1ºC in a water bath.

Egyptian Mallow was cleaned and dry leaves which were removed, followed by washing and blanching in the same three different solutions for 1.5 min at 98 ± 1 ºC and then subjected to steam blanching under atmospheric pressure for another1.5 min.

Green beans were left to cool for 2min., drain and then frozen in a fluidized bed freezer. Samples were frozen at an average air temperature of at –35 ± 3 ºC, and the fruit temperature was recorded with a thermocouple placed in the centre of a green bean test sample. Approximately 250 g of frozen green beans were immediately put into polyethylene bags and heat sealed. Packed frozen green beans were stored in storage chamber at –18 and –12 ±2 ºC for 6 months in Basma Company from local market at Obour city, Egypt.

Egyptian mallow leaves were blanched by passing over a long wire mish belt for chilling at 5 to 15 ºC. The leaves were than squeezed and shopped into small pieces (2-3 mm) and packing into polythene bags (250 g/ bag) and heat sealed. The bags were pressed tightly to remove as much air as possible, then sealed closely and finally frozen at – 35 ± 3 ºC in a tunnel freezer and stored in storage chambers at –18 ± 2ºC and –12 ±2 ºC for 6 months in Basma Company from local market at Obour city.

Methods:

Chemical analysis

Determination of pH

The pH values were measured using a pH meter (HANNA-Instrument, USA) according to the **(AOAC 2000).**

Determination of Ascorbic Acid

Ascorbic Acid (Vitamin C) content was determined by the 2, 6- dichlorophenol indophenol titrimetric methods **(Renganna, 1979).**

Determination of total chlorophyll

The determination was carried out according to the method reported by **Holden (1965)** as follow: The fresh sample (0.5 g) was grinded in a mortar with acetone in presence of little amount of calcium carbonate then filtered. The residue was washed with acetone several times until the washing liquid become colorless. The extract and washing were made up to a known volume (50ml). The absorbance was measured by a Spectrophotometer JENWAY Model 6105 England at 663 and 645 nm for cholrophyl a and b, respectively in 1cm cuvett cells assuming an 80 % aqueous acetone as blank.

The concentration was calculated from the following equation:

Chlorophyll a (mg/g) = $(12.3 \times A_{663} - 0.86 \times A_{645} \times V) / a \times 100 \times W$ -------- (1)

Chlorophyll b (mg/g) = (19.3 × A_{645} - 3.6 × A_{663} × V) / a ×100 ×W --------- (2)

Where: A_{645}, A_{663} are the absorbance of 645, 663 nm, V is volume in ml, a is the length of light path in cell and W is the fresh weight in gram.

Determination of Enzymatic activities

The method described by **Angela et al., (2001)** was applied to prepare the crude enzyme extracts. In this method, leaf tissues were homogenized in a chilled pastel and mortar with 100ml potassium phosphate buffer (pH 7.5) containing 1ml EDTA, 3 ml DL-dithiothreitol and 5% (W/V) insoluble polyvinylpyrrolidone (3:1; buffer volume : tissue fresh weight). The homogenates were centrifuged at 10.000 rpm for 30 min at 4ºC. and the supernatants were kept in separate aliquots at – 4ºC until analysis.

Peroxidase (POD) activity: was assayed by photochemical method as described by **Amako et al., (1994)** using pyrogallol as substrate and the absorbance was measured at 430nm.

Polyphenol Oxidase (PPO) activity: was assayed photochemically as described by **Coseteng and Lee (1987)** by using catechol as substrate and the absorbance was measured at 420nm.

Lipoxygenase (LOX) activity: was assayed by using linoleic acid as substrate as described by **Surry (1964).** The absorbance was measured at 234nm over a period of 5 min.

Calculation of kinetic constant (k)

Degradation rate constants of quality parameter including vitamin C, chlorophy and enzymatic activities) were calculated by multiplying the value of the slopes of the regression lines by 2.303. The regression lines were obtained by plotting the logarithms of quality parameter remaining in green beans & Egyptian mallow as a function of storage time and blanching solution for all temperature studied (-18 and -12±2ºC). The concentrations of vitamin C, chlorophyll a, chlorophyll b and enzymes activity as a function of time at a constant temperature were modelled by first-order degradation kinetic [Eq. (3)] **(Koca et al., 2006):**

$Ln (C/C_0) = - kt$; ---------- (3)

Where: C is the concentration at any time t, C_0 is the initial concentration, and k is the first-order rate constant ($month^{-1}$).

Calculation of half-life time values ($t_{½}$)

The half-life time values of quality parameter degradation were calculated using the equation [Eq. (4)] as follows **(Koca et al., 2006):**

$t_{½} = \ln 2/k$ ---------- (4)

Where: k is the rate constant ($month^{-1}$).

Calculation of activation energy (Ea)

Temperature dependence of quality parameter degradation was determined as mentioned by **Giannakourou and Taoukis (2003)** using Arrhenius equation [Eq. (5)] as follows:

$k= k_{ref} . \exp [– Ea / R (1/T-1/T_{ref})]$ ------- (5)

Where: Ea is the activation energy of the chemical reaction (J mol^{-1}), k is the rate constant, k_{ref} is the reaction rate of the studied parameters at a reference temperature T_{ref}, R universal gas constant (J $mol^{-1} K^{-1}$), and T is the absolute temperature (K). By linearly correlating ln K vs ($1/T-1/T_{ref}$), the Ea of the chemical reaction was estimated from the slope of the fitted line.

Color measurement

Color was measured using a Hunter-Lab Model [MOM BUDAPEST MOMCOLORD], and was expressed in L*, a* and b* Hunter scale parameters then a* and b* were used to compute hue angle (tan_1b*/a*) as reported by **Gonçalves et al., (2009)**.

Sensory evaluation

Samples were evaluated according to the numerical scoring test **(Ranganna, 1979)**. Ten panelists were evaluated each sample on a specific scale for color, flavor, texture and overall

Statistical analyses

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at 5% level of significance was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System **(SAS, 1996)**.

3. Results and Discussion

Kinetic study of vitamin C loss

Vitamin C is an important anti-oxidant in the human body that can prevent cancer**.** Vitamin C losses occur when DHAA is oxidised to 2,3-diketo-L-gulonic acid. Due to its sensitivity, vitamin C has been considered a good indicator of nutritional quality of water soluble vitamins in frozen vegetables **(Martins and Silva, 2003).**

Vitamin C retention in the frozen green bean and Egyptian mallow samples were illustrated in **Fig 1,** just after freezing process until the end or freezing storage. In all cases, vitamin C loss was found to be adequately described by an apparent first order reaction [Eq. (3)]

After the freezing/blanching process, during the subsequent isothermal frozen storage, green bean and Egyptian mallow exhibited a first order loss of vitamin C at all blanching solutions studied. Data in **Table 1** show kinetic parameter (K, Ea, $t_{1/2}$) of the investigated green bean and Egyptian mallow samples at storage temperatures of -12 and -18ºC.

Temperature dependence of vitamin C loss was expressed using Arrhenius equation, and the estimated activation energies, Ea, the 95% confidence range as well as the goodness of fit (R^2) for frozen green bean showed loss in vitamin C content with time and

temperature due to its sensitivity and affection by the oxidation-reduction potential of the reaction medium. The obtained results are in agreement with **Serpen and Gökmen, (2007)**. Data in **Table 1** illustrated that water blanching and 0.2% NH_2HCO_3 blanched samples showed k-values of 0.104 and 0.185 after 6 months at -18°C and the vitamin C degradation rates were higher for the higher storage temperature (-12°C).

Shelf life values for frozen green bean and Egyptian mallow could lead to different estimations, which may be attributed to different treatments of blanching that could affect the mode of deterioration, due to different enzyme concentration, water activity and other factors. Apart from the previous analysis, an alternative approach for modelling the temperature dependence of the deterioration reaction was also applied for green bean, which gives an adequate fit (R^2=0.97) for blanching in water and in 0.1 % $MgCO_3$ at -12°C but in 0.2% NH_2HCO_3 the R^2-value was 0.947, while for Egyptian mallow the best R^2 was 0.996 for blanching treatment in 0.1% $MgCO_3$ solution.

The half-life values (t1/2) were higher at -18ºC in all samples than at -12ºC. Data in Table (1) clearly demonstrate that the half-life value was (6.66 month) in water blanching, while it was 5.97 and 3.75 months for blanching in 0.1% $MgCO_3$ and 0.2% NH_2HCO_3 respectively. The same trend was noted for Egyptian mallow sample. **Martins and Silva (2003)** reported that, improper frozen storage causes evident changes in sensory characteristics that can influence consumer acceptability and leads to products of reduced nutritive value, mainly in vitamin C.

Chlorophyll a and b losses

The blanching treatment and freezing process did not significantly change the initial chlorophyll content in studied vegetables as seen in **Fig 2.** Also, as expected, green beans exhibit an initial higher content of chlorophyll a than b. The same trend was noticed in Egyptian mallow. The low variability in chlorophyll content of green bean was due to the mixing samples while doing chlorophyll extraction. Chlorophylls a and b were observed during the 180 days of frozen storage for the two studied temperatures (-12 and -18ºC). The degradation of both chlorophyll a and b followed a first order reaction kinetics with Arrhenius behaviour at the different storage temperature, exhibiting a good R^2 in experimental data **Table 2.**

Low differences were obtained between the degradation rates of chlorophyll a and b, as was previously reported during blanching treatment experiments **(Heaton et al., 1996)**. Because of the low variation in chlorophyll a and b content, low variation and co-linearity between the estimated kinetic parameters was obtained leading to excellent precision in both kinetic rate at the reference temperature and Arrhenius activation energies **Table 2**.

The high scores of the studied effect of the kinetic parameters emphasise that all parameters are important to describe chlorophyll loss during storage. Furthermore, they demonstrate the applicability of the Arrhenius law. The high activation energy of chlorophyll degradation enables frozen storage to kinetically constrain the pigment destruction, which makes frozen storage at low temperatures a good mean for preserving chlorophyll. However, the high activation energy also makes this quality parameter very sensitive to higher storage temperatures and temperature fluctuations during frozen storage.

Activation energies were calculated on the basis of linear regression analysis of natural logarithms of rate constants (K-values) at different blanching solution values against reciprocal absolute temperature, 1/T (in K). The slopes of linear regression lines resulted in apparent activation energies of 142.36, 102.01 and 92.57 kJ/mol for chlorophyll a in green bean blanched in water, 0.1% $MgCO_3$ and 0.2% NH_2HCO_3 respectively, while the chlorophyll a degradation recorded the adverse reaction in Egyptian mallow sample. On the other hand, the half life (t1/2) recorded the highest values at -18ºC in both green bean and Egyptian mallow for chlorophyll a and chlorophyll b.

The rate constants of green colour loss decreased with increasing pH, indicating that the green colour was retained at higher pH conditions as seen in **Fig 3**.

On the other hand, it seems that the higher pH value ranged from 6.89 to 7.45 in both samples did not change the rate of color loss at different blanched solutions. **Gunawan and Barringer (2000)** found no significant difference in color change between pH 7 and 8 within the time limits of the experiment. In addition, **Sweeney and Martin (1961)** determined no further decrease in chlorophyll retention above pH 6.8 and revealed that chlorophyll degradation should occur by the same mechanism at the higher pH but the rate would be so slow that it would require a much longer time frame to study.

Heat stabilities of POD, PPO and LOX

Conventionally, the vegetables are blanched to the point of destruction of POD activity and it is generally accepted that if POD is destroyed then it is quite likely that other enzymes will not survive. In this study, three separate solution blanching operations were performed at 96ºC for 2.5 min for green bean and for Egyptian mallow samples blanched in tap water at 98ºC for 1.5 min followed by steam blanching for another 1.5min to achieve 90% inactivation of initial POD, PPO and LOX activities, respectively as seen in **Table 3.** Blanched green bean and Egyptian mallow were then subjected to a storage

test at -12 and -18ºC up to 6 months to re-evaluate the use of POD, PPO and LOX as indicator enzymes for optimizing the blanching conditions.

The regeneration of POD activity after heat treatment was reported by some researchers in model conditions **(Rodrigo et al., 1997)**, but not encountered during frozen storage of vegetables **(Gokmen et al., 2000)**. Our results showed that no regeneration of POD, PPO and LOX activity was detected in any blanched samples during storage at -18ºC in green bean and Egyptian mallow **Table 3,** while POD activity continued to decrease through storage period. These results showed that blanching treatment at 96ºC for 2.5 min for green bean samples and Egyptian mallow blanched in tap water at 98ºC for 1.5 min, followed by steam blanched for another 1.5 min were sufficient to inactivate LOX, POD and PPO, respectively.

The obtained results for green bean and Egyptian mallow were also satisfactorily described by an Arrhenius first-order kinetic model for all temperatures tested (an example of model fit obtained by one-step regression analysis is also included in **Table 3**. The quality of the model fit was assessed by analyses of the residuals (*i.e.* normality and randomness were confirmed, R^2 was above 0.854). Estimated kinetic parameters and confidence intervals at 95% are also included in **Table 3** value of E_a 88.23 kJ/mol was recorded for blanching in 0.2% NH_2HCO_3. There is often a lack of information concerning the precision of those estimates. For example **Bifani et al., (2002)** referred that the value of 99.1 kJ/mol for peroxidase inactivation in green beans.

For optimum quality retention of vegetables during frozen storage, it is recommended a reduction of 90% of the peroxidase activity after the blanching treatment **(Bahceci et al., 2005)**. The enzymatic activities were little temperatures dependant (lower activation energy) than the chemical indices, and hence color change will be the dominant reason for rejection at inadequate storage of -12ºC or higher as indicated by the experiments.

Color characteristics change

Color differences were perceptible to the human eye between green bean samples, stored at different temperatures, since the first measurement. The results of colour differences (Δ E) revealed that blanching in alkali media caused in a significant decrease in Δ E (reached to 4.55) than blanching in water (5.10). The loss of color occurred during storage and was faster at the high storage temperatures of -12ºC, attaining great color differences after 6 months of storage which was 12.02, 10.29 and 8.08 for water, 0.1% $MgCo_3$ and 0.2% NH_2HCO_3 blanched green beans, as compared with the other samples were stored at -18ºC, with scores of 11.25, 7.98 and 7.13, respectively, as shown in **Table 4.**

All green bean samples blanching in 0.2% NH_2HCO_3 were greener and showed less color change from the water blanched particularly, under storage at -18ºC. The change in hue value was attributed to the degradation of chlorophyll pigments from bright green to olive green and finally to yellow due to the chlorophyll's pheophytisation **(Lau et al., 2000).**

It is evident from **Table 4** that a-values are higher for blanched Egyptian mallow in 0.1% $MgCo_3$ and 0.2% NH_2HCO_3 solutions, indicating that there is some stabilization of green color containing salt. This beneficial effect is continues through freezing and storage. Also **Shalini et al., (2008)** reported that degree of greenness is important for determining the final quality of thermally processed leafy vegetables, which acquire their green color due to chlorophyll. The color change from bright green to olive brown during processing is attributed to conversion of chlorophylls to pheophytins due to the loss of central magnesium ion. The results illustrated in blanched Egyptian mallow indicated that there is a loss in color as can be seen from the decrease in Hunter L and a-values in all the samples. Under storage at -18ºC, Egyptian mallow blanched in 0.2% NH_2HCO_3 shows maximum retention of green color as compared to the blanched in water, 0.1% $MgCo_3$ solution, and the storage at -12ºC accelerates green color losses in all treated Egyptian mallow samples.

Sensory evaluation of the suggested samples

The preservation of the sensory characteristics of food is usually a guarantee of its microbiological quality. In fact, the spoilage of a foodstuff's color, its odor and/or texture, though partly due to biochemical processes, is accompanied by an excessive microbial growth that is also implicated in this alteration **(Giménez et al., 2003)**. In present study, the sensory evaluation tests showed the mean color score for the 0.2% NH_2HCO_3 treated samples being judged greener, described by substantially reduce the transformation of chlorophyll into pheophytin by addition of NH_2HCO_3 in blanching medium and continuing this beneficial effect through frozen storage. In order to have a higher rating of flavor and total acceptability than the water and 0.1% $MgCO_3$ treated samples by the panel after 6 months of frozen storage. As regards to the overall visual quality, all samples showed a deteriorated trend during frozen storage as **Table 5** indicates that, blanching of green bean by 0.2% NH_2HCO_3 led to maintain the overall quality, i.e. 8.5 ± 0.6^{ab} after 6 months storage at -18ºC followed by 0.1% $MgCO_3$ treatment (8.4 ± 0.5^{ab}) but water blanched samples had poorer overall visual quality than other samples (7.2 ± 0.9^{d}); however, the visual quality of green bean deteriorated more rapidly at -12ºC (in the

same Table) than at -18ºC after 6 months of storage, the acceptability of green bean treated by 0.2% NH_2HCO_3 was significantly better than that of green bean treated by water and 0.1% $MgCO_3$ as a blanching media. Thus, color of green bean was maintained during storage as indicated by panel scores and chemical analysis of chlorophyll a and total chlorophylls. So the color change of frozen green bean was dependent of storage time and its pH value. The color quality was used as a key factor to determine the shelf-life of frozen green bean.

Data in **Table 5** showed the organoleptic parameters used for evaluating frozen Egyptian mallow which blanching in 0.2% NH_2HCO_3 and stored for 6 months at -18 ºC. The panellist's record fancy grade for the Egyptian mallow samples in all parameters, the flavour and acceptability had (9.0 ± 0^a) for the Egyptian mallow samples. On the other hand, the data proved that the Acceptability of the suggested Egyptian mallow sample reached a value of (8.8 ± 0.3^a) stored at -12 ºC and thus lead within the fancy category. Statistical analysis of the Egyptian mallow samples stored for 6 months at -12 ºC proved the presence of significant differences between color, flavor, texture and acceptability. Sensory evaluation comprises a set of techniques for accurate measurements of human responses to foods and minimizes the potentially biasing effects of brand identity **(Klimczak et al., 2007).**

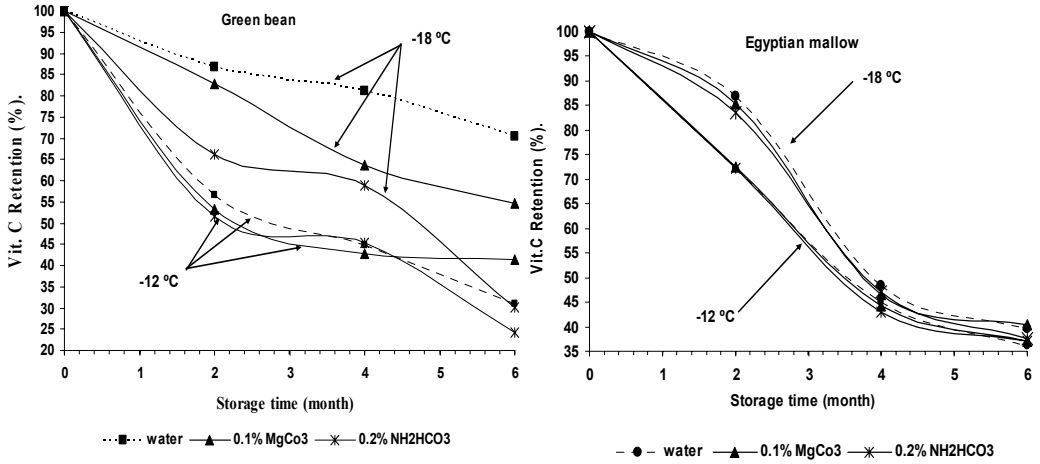

Fig 1. Vitamin C retention (%) of frozen green bean and Egyptian mallow samples during storage for 180 days at -12 and -18ºC.

Table 1. Kinetic parameters for vitamin C degradation in blanched green bean and Egyptian mallow in different solution

Kinetic parameters	Blanching solution					
	Water		0.1% $MgCo_3$		0.2% NH_2HCO_3	
	-12ºC	-18ºC	-12ºC	-18ºC	-12ºC	-18ºC
Green bean at 96ºC						
$K^{month-1}$	0.214	0.104	0.188	0.116	0.218	0.185
R^2	0.970	0.990	0.970	0.958	0.947	0.929
Ea kJ/mol	135.13	135.13	118.70	118.670	101.99	101.99
($t_{1/2}$) month	3.24	6.66	3.69	5.97	3.18	3.75
Egyptian mallow at 98ºC						
$K^{month-1}$	0.178	0.169	0.173	0.172	0.171	0.173
R^2	0.937	0.910	0.996	0.916	0.988	0.968
Ea kJ/mol	94.85	94.85	92.38	92.38	91.45	91.45
($t_{1/2}$) month	3.89	4.10	4.00	4.03	4.00	4.05

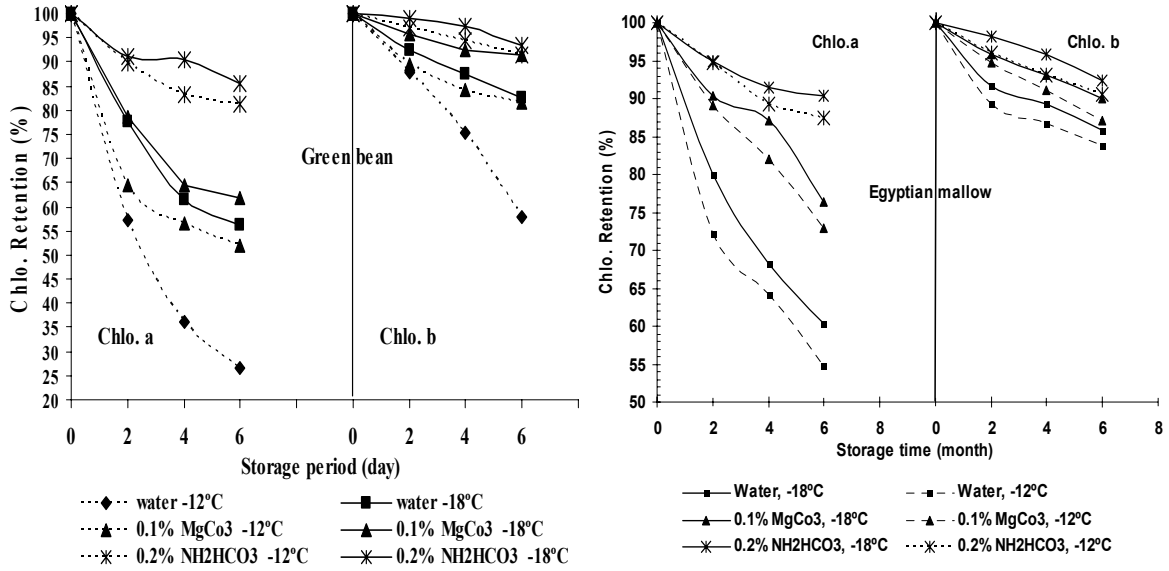

Fig 2. chlorophyll content as (mg/100 g) of frozen green bean and Egyptian mallow during storage for 180 days.

Table 2. Kinetic parameters for Chlorophyll a and b degradation in green bean and Egyptian mallow blanched in different solutions.

Kinetic parameters	Blanching solution					
	Water		0.1% $MgCo_3$		0.2% NH_2HCO_3	
	-12ºC	-18ºC	-12ºC	-18ºC	-12ºC	-18ºC
Green bean						
Chlorophyll a						
K $^{month-1}$	0.223	0.098	0.105	0.082	0.035	0.024
R^2	0.984	0.964	0.865	0.929	0.931	0.908
Ea kJ/mol	142.36		102.01		92.57	
($t_{1/2}$) month	3.11	7.07	6.60	8.45	19.80	28.88
Chlorophyll b						
K $^{month-1}$	0.090	0.031	0.034	0.015	0.015	0.011
R^2	0.972	0.990	0.935	0.949	0.998	0.916
Ea kJ/mol	132.62		113.60		98.88	
($t_{1/2}$) month	7.70	22.36	20.39	46.21	64.21	63.01
Egyptian mallow						
Chlorophyll a						
K $^{month-1}$	0.107	0.094	0.041	0.032	0.023	0.017
R^2	0.923	0.958	0.968	0.878	0.964	0.929
Ea kJ/mol	97.39		99.23		99.45	
($t_{1/2}$) month	6.48	7.37	16.91	21.66	30.14	40.77
Chlorophyll b						
K $^{month-1}$	0.028	0.024	0.023	0.017	0.016	0.013
R^2	0.885	0.931	0.996	0.994	0.994	0.974
Ea kJ/mol	96.05		99.45		96.71	
($t_{1/2}$) month	24.75	28.88	30.14	40.77	43.22	53.32

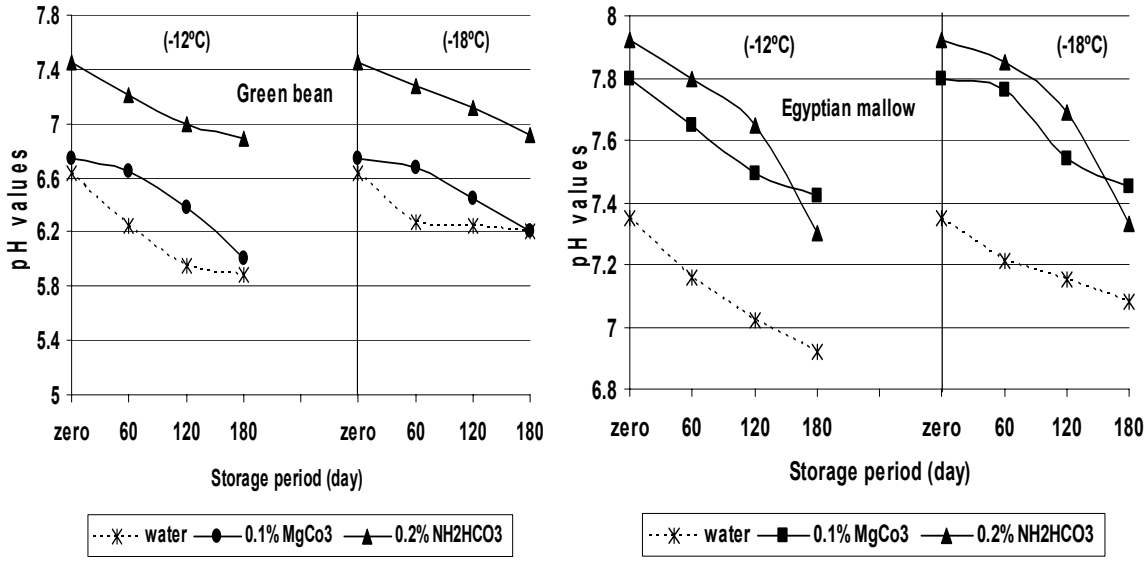

Fig 3. Chlorophyll degradation at different pH values for frozen green bean and Egyptian mallow during storage for 180 days.

Table 3. Kinetic parameters for enzymes activity degradation in blanched green bean and Egyptian mallow in different solution

Kinetic parameters	Blanching solution at 96ºC					
	Water		0.1% $MgCo_3$		0.2% NH_2HCO_3	
	-12ºC	-18ºC	-12ºC	-18ºC	-12ºC	-18ºC
Green bean						
Peroxidase (POD) activity						
$K^{month-1}$	0.194	0.179	0.194	0.159	0.165	0.156
R^2	0.988	0.996	0.988	0.974	0.974	0.994
Ea kJ/mol	87.80		82.12		89.30	
Polyphenol Oxidase (PPO) activity						
$K^{month-1}$	0.223	0.176	0.160	0.154	0.136	0.129
R^2	0.945	0.962	0.904	0.937	0.985	0.923
Ea kJ/mol	79.52		90.16		89.70	
Lipoxygenase (LOX) activity						
$K^{month-1}$	0.272	0.241	0.210	0.186	0.191	0.176
R^2	0.964	0.966	9.972	0.962	0.998	0.996
Ea kJ/mol	84.25		85.46		87.73	
Egyptian mallow						
Peroxidase (POD) activity						
$K^{month-1}$	0.181	0.143	0.145	0.120	0.153	0.141
R^2	0.918	0.865	0.994	0.895	0.945	0.895
Ea kJ/mol	80.95		83.87		88.23	
Polyphenol Oxidase (PPO) activity						
$K^{month-1}$	0.114	0.079	0.083	0.083	0.101	0.101
R^2	0.990	0.976	0.978	0.978	0.970	0.970
Ea kJ/mol	78.76		81.94		89.31	
Lipoxygenase (LOX) activity						
$K^{month-1}$	0.164	0.134	0.145	0.124	0.129	0.990
R^2	0.974	0.970	0.904	0.854	0.970	0.990
Ea kJ/mol	82.88		85.20		81.53	

Table 4. Influence of different blanching media and storage temperature on color characteristics of green bean and Egyptian mallow**.**

Item	Treatments	Green Bean blenched in			Egyptian mallow blenched in		
		Zero time	After 6 months of frozen storage at		Zero time	After 6 months of frozen storage at	
			-18 ± 2 ºC	-12 ± 2 ºC		-18 ± 2 ºC	-12 ± 2 ºC
L	Fresh	41.75			29.22		
	water	40.61	38.86	36.20	24.70	22.86	21.47
	0.1% $MgCo_3$	38.29	35.39	34.81	23.82	21.93	20.75
	0.2% NH_2HCO_3	37.82	34.69	32.95	23.39	21.04	20.01
a	Fresh	-23.73			-15.32		
	water	-16.15	-13.36	-13.14	-11.88	-11.39	-11.27
	0.1% $MgCo_3$	-18.72	-16.31	-14.39	-12.54	-11.96	-11.35
	0.2% NH_2HCO_3	-21.69	-20.09	-19.21	-13.48	-12.82	-12.13
b	Fresh	28.15			19.97		
	water	23.09	16.41	14.54	16.58	13.18	11.27
	0.1% $MgCo_3$	24.01	20.48	19.02	17.18	14.66	12.16
	0.2% NH_2HCO_3	24.62	21.60	19.93	17.74	16.44	13.75
Δ E	Fresh	---			---		
	water	5.10	11.25	12.02	18.00	20.36	22.46
	0.1% $MgCo_3$	4.56	7.98	10.29	16.87	18.15	20.11
	0.2% NH_2HCO_3	4.55	7.13	8.08	16.5	17.53	19.56
Chroma	Fresh	36.33			27.58		
	water	28.68	21.16	19.60	21.05	18.42	17.16
	0.1% $MgCo_3$	29.90	27.07	24.58	22.13	20.92	19.71
	0.2% NH_2HCO_3	31.31	28.69	27.54	23.18	22.06	21.18
Hue angle (tan^{-1} b*/a*)	Fresh	-67.59			- 63.94		
	water	-60.84	-45.55	-43.89	- 57.41	- 48.17	- 45.63
	0.1% $MgCo_3$	-63.07	-50.85	-48.67	- 59.46	- 48.94	- 46.21
	0.2% NH_2HCO_3	-64.01	-54.17	-52.94	- 60.49	- 50.79	- 47.83

L = lightness; a = greenness; b = yellowness; Δ E = color difference.

Table 5 Sensory evaluation of green bean and Egyptian mallow blanched in different solution and stored at -18, -12 ºC for 6 months.

Blanching solution	Organoleptic parameters			
	Colour (10)	Flavor (10)	Texture (10)	Acceptability (10)
Green bean storage at -12ºC				
Water	5.5±0.9^{c}	5.7±1.1^{e}	6.7±1.1^{b}	6.2±0.5^{e}
0.1%$MgCO_3$	7.2±1.0^{b}	7.0±1.0^{d}	6.4±0.6^{b}	6.8±0.5^{d}
0.2% NH_2HCO_3	8.6±0.5^{a}	8.0±0.9^{bc}	6.3±1.8^{b}	8.0±0.7^{c}
Green bean storage at -18ºC				
Water	6.8±1.0^{b}	6.9±1.2^{d}	7.9±0.8^{a}	7.2±0.9^{d}
0.1%$MgCO_3$	8.7±0.9^{a}	8.4±0.9^{ab}	7.0±1.0^{b}	8.4±0.5^{ab}
0.2% NH_2HCO_3	8.7±0.9^{a}	8.4±0.9^{ab}	7.0±1.0^{b}	8.5±0.6^{ab}
Egyptian mallow storage at -12ºC				
Water	7.1±0.9^{b}	5.8±1.3^{e}	8.5±0.5^{a}	6.9±0.4^{d}
0.1%$MgCO_3$	8.1±0.5^{a}	7.6±1.0^{bcd}	8.5±0.5^{a}	8.1±0.4^{bc}
0.2% NH_2HCO_3	8.5±0.5^{a}	8.4±0.5^{ab}	8.7±0.4^{a}	8.8±0.3^{a}
Egyptian mallow storage at -18ºC				
Water	8.2±1.0^{a}	7.1±0.9^{cd}	8.6±0.5^{a}	7.9±0.2^{c}
0.1%$MgCO_3$	8.6±0.5^{a}	8.4±0.5^{ab}	8.7±0.4^{a}	8.9±0.2^{a}
0.2% NH_2HCO_3	8.7±0.4^{a}	9.0±0^{a}	8.8±0.4^{a}	9.0±0^{a}

Data are presented as means ± SDM (n= 10) & Means within a column with different letters are significantly different at (P≤ 0.05)

Conclusion

From the obtained results, in both green bean and Egyptian mallow blanching should be applied before freezing. Ascorbic acid is an important criteria for quality of these vegetables, which can be oxidized easily with several factors. Also both of color and sensory evaluation are limiting factors.

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