A system using in situ NIRS sensors for the detection of product failing to meet quality standards and the prediction of optimal postharvest shelf-life in the case of oranges kept in cold storage

Dolores Pérez-Marín [a,*], Luis Calero [b], Tom Fearn [c], Irina Torres [b], Ana Garrido-Varo [a], María-Teresa Sánchez [b,*]

[a] *Department of Animal Production, University of Cordoba, Campus of Rabanales, 14071 Córdoba, Spain.*

[b] *Department of Bromatology and Food Technology, University of Cordoba, Campus of Rabanales, 14071 Córdoba, Spain.*

[c] *Department of Statistical Science, University College London, WC1E 6BT, United Kingdom*

**Corresponding authors*. Tel.: +34 957 212576; fax: 34 957 212000

E-mail addresses: teresa.sanchez@uco.es (M.T. Sánchez) or dcperez@uco.es (D. Pérez-Marín).

Summary

The viability of using near infrared (NIR) spectroscopy was studied as a non-destructive analytical technique with the potential of being applied *in situ* to establish quality standards and the postharvest shelf-life of oranges kept in cold storage, as well as to detect substandard produce. In specific terms, it was applied to assessing the viability of increasing the period of postharvest storage depending on the quality exhibited by the produce. Initially, the spectral information from 80 oranges stored for up to four weeks in refrigeration chambers was used, this being the maximum postharvest storage time in the citrus industry in the south of Spain, to establish the natural variability in spectra from refrigerated oranges meeting quality standards. The processing of the spectral data was carried out using principal component analysis and the spectral distances between the sets (fruit belonging to weeks 1 to 4 of cold storage) were calculated using n-dimensional statistics such as the Mahalanobis distance. Subsequently, oranges stored for between five and ten weeks were spectrally analysed and their distances from the standard or control population, described above, were calculated. The results were represented in the form of a Shewhart control chart, in which the mean scores and the corresponding control limits serving as warning systems were established. The findings suggest that NIR spectroscopy and the use of spectral distances will enable an innovative quality control system to be developed, based on spectral information that allows the establishment of quality standards in oranges, and the detection of non-standard produce.

Keywords: NIR spectroscopy; Orange; Postharvest storage; Quality standards; Shewhart control chart; Substandard produce.

1. Introduction

At present the quality control and traceability of oranges is exclusively based on destructive pre- and postharvest analyses in the laboratory on a number of samples per batch, despite the high degree of variability in the produce (Obenland et al., 2009; Kallsen et al., 2011). Although the traditional physical-chemical methods are accepted for determining the quality of citrus fruit, they involve a series of disadvantages that need to be borne in mind; primarily being destructive, time consuming and they do not enable analysis to be carried out when the fruit is ripening on the tree or in postharvest cold storage. Moreover, the samples chosen may not be representative of the quality of the consignment received by the industry, given the variability exhibited by oranges even within the same variety and batch.

Once the oranges have been picked, the freshly-processed citrus industry usually carries out postharvest storage of the fruit in refrigerated conditions at a temperature of 3-8°C, depending on the cultivar, fruit maturity and the production area (Arpaia and Kader, 1999).

Obenland et al. (2008) point out that during the postharvest cold storage of oranges, their soluble solid content (SSC) increases while their titratable acidity (TA) decreases, giving rise to an increase in the ripening index (SSC/TA) as the time in cold storage is extended. The same authors report that the evaluations of tasting panels indicated that the “fresh” flavour of oranges diminishes progressively as a result of such storage. In addition, fruit held in cold storage (5 ºC, HR: 85-90%) for three weeks exhibit tighter peel compared to those that have just been harvested (0 weeks of cold storage) or those stored for six weeks at 5 ºC and a relative humidity of 85-90%.

For the citrus industry in general and the fresh fruit industry in particular, it is extremely important not only to classify fruit in terms of their quality upon delivery but also to have the ability to establish product quality standards and rapid and accurate automated systems to control the quality. This is necessary in order that the fruit always exhibits optimal and homogeneous characteristics, enabling batches to be accepted or rejected on the basis of such quality in a matter of seconds, as well as establishing the maximum period of cold storage that enables this standard to be maintained. To realise this a rapid and non-destructive analytical technologies that are not limited of cost or analysis time should be used which will enable decisions to be taken and actions implemented in real time, aimed at ensuring the quality of citrus fruit and the approval of batches, in terms not only of the external appearance but also internal quality.

NIR spectroscopy currently provides one of the most practical ways of meeting such requirements, since it is non-invasive and combines speed, ease of use and highly accurate measurements with low analysis costs and considerable versatility (Nicolaï et al., 2007). This enables its use at various levels of decision-making, both in the field, prior to harvesting and subsequently, in the industrial setting, allowing postharvest decisions to be taken concerning the quality and shelf-life of fresh produce during its cold storage (Sánchez and Pérez-Marín, 2011).

This technology has already been successfully applied in the compound feed industry for the determination of quality control standards in accordance with the quality requirements established for the different raw materials comprising the feeds in question (Montoya et al., 2013); hitherto there have not existed any applications for establishing quality control tests in the citrus industry.

Process control is nowadays an indispensable tool in overseeing processes carried out in the agri-foods industry, once such process being the postharvest preservation of fruit in cold storage. One of the oldest process control tools is the Shewhart chart (Shewhart, 1931) in which statistics derived from measurements on the process are plotted in time sequence on a chart that has limits defining the variability expected from an in-control process. These limits come from the assumed distribution of the statistic, often but by no means always a normal distribution. The application of tools such as Shewhart control charts enables compliance testing to be conducted and substandard produce do be identified, facilitating quality control and the process monitoring. One main advantage of Shewhart control charts is the ability to identify anomalous variability in the process to be reliably identified, thereby contributing to enhancements in quality (Gejdoš, 2015). They also offer a more flexible tool for dealing with any non-compliant produce that is encountered, because the spectrum provides comprehensive information about the product, encompassing highly diverse aspects related to quality (Montoya et al., 2013).

The use of NIR sensors designed for *in situ* applications enables real-time decision-making systems to be installed in the food chain, improving the productivity and quality control of the products in question (Sánchez et al., 2012, 2017; Torres et al., 2016; De la Roza-Delgado et al., 2017; Zhang et al., 2017). This *in situ* control, much needed in the fresh orange sector, is made possible thanks to two characteristics of the recent developments in NIR instrumentation: miniaturisation and portability.

Such sensors have thus been used to determine the quality of oranges on the tree (Sánchez et al., 2012; Torres et al., 2016). No NIRS studies have been found in the scientific literature however that address the application of this technology to determine either the compliance of batches with the quality criteria set out in legislation or by the fresh fruit-handling industry itself, or the postharvest shelf life in cold storage in a way that is designed to ensure such standards.

The goal of the present research is to develop a methodology involving the *in situ* use of portable NIR sensors to establish a quality control system for oranges kept in cold storage based exclusively on spectral information, and to determine the optimal duration of postharvest cold storage for these fruits, with the aim of complying with the standards and despatching the produce with homogeneous characteristics.

2. Material and methods

2.1. Sampling

190 oranges (*Citrus sinensis*, L. cv. 'Navelina'), grown in Palma del Río (Córdoba, Spain), were picked at commercial maturity on 10 January 2017. The oranges were taken to the premises of Zamefruit, S.L.L. (Palma del Río, Córdoba, Spain) where they were industrially processed (washing and disinfection, waxing and size sorting) and placed in cold storage at 4 ºC and 90% RH, for a maximum storage period of 10 weeks, and subjected to a weekly sampling process (20 oranges per week, except the eighth week, in which 10 samples were analysed).

During cold storage, all the oranges were weighed on a weekly basis and given a visual examination in order to detect possible disorders.

The oranges were subjected to both a spectral and a physical-chemical analysis at the laboratories of the University of Córdoba. Prior to the spectral acquisition and the physical-chemical analyses, the oranges were equilibrated to room temperature (20 ºC).

2.2. NIRS spectral acquisition

For the purposes of acquiring the NIR data of the intact oranges a Phazir 2400 (Polychromix, Inc., Wilmington, MA, USA) was used in reflectance mode. This is a compact and manual instrument, with a built-in DTS-NIR spectrophotometer based on micro-electro-mechanical system (MEMS) technology and a tungsten light source to illuminate the sample in the near infrared region. The reflected light is collected and measured using a single InGaAs photodetector, and the instrument has no moving parts. The spectrophotometer scans in a non-constant interval of 8 nm, over a range of wavelengths covering 1600-2400 nm. The integration time of the sensor is 600 ms. The MEMS device measures an area of approximately 4 mm^2 and is equipped with quartz protection to prevent dirt from entering and to facilitate cleaning of the contact area.

For the NIR spectral readings, four measurements were carried out at the equator of each fruit, located 90º from each other. The four spectra were averaged to obtain a mean spectrum per fruit.

2.3. Reference data

Individual oranges were weekly weighed using an electronic balance (0-1,000 ± 0.01 g; P1000 N, Metter-Toledo, GmbH, Greifensee, Switzerland). The firmness of the fruit was determined as the resistance of the peel and the pulp to penetration, according to the Magness-Taylor method with a Universal Testing Machine (model 3343, single column, Instron, Norwood, MA, USA). The velocity was set at 0.0016 m/s (100 mm/min), using a load cell of 1000 N. The firmness was defined as the force necessary to penetrate an orange to a depth of 10 mm, using a 6 mm diameter probe. The fruit was placed with the peduncle-calyx axis in a horizontal position for two measurements, the first in a position around the equator of the fruit, and the second having turned it

180º. Thereafter the oranges were individually squeezed using a domestic juicer, to determine SSC and TA in accordance with Obenland et al. (2008). BrimA was calculated using the equation established by Jordan et al. (2001):

$$BrimA = SSC - k(TA)$$

where k is a constant that reflects the greater sensitivity of the tongue to TA compared to SSC. K was assigned a value of 4, in accordance with Obeland et al. (2009).

All the samples were analysed in duplicate and the standard error of laboratory (SEL) was estimated from these duplicates.

2.4. Processing the spectral and reference data and constructing the Shewhart control charts

To determine the optimal duration of postharvest cold storage for oranges and the quality parameters that have the greatest impact on the postharvest shelf-life, a methodology based on Shewhart control charts (Sanusi et al., 2017) was used, based on the values of spectral distances (Mahalanobis distance, GH) and also the reference values exhibited by the quality parameters: weight, firmness, SSC, TA and BrimA.

First, following the procedure set out by Montoya et al. (2013), a quality standard for oranges kept in cold storage (4 ºC; 90% RH) was spectrally defined using principal component analysis (PCA); this comprised oranges kept in cold storage for a maximum duration of four weeks (N = 80 samples), the typical postharvest storage time for fruit among companies handling fresh oranges in the south of Spain. Next, the standard was spectrally compared to the one exhibited by the rest of the oranges kept in cold storage for a maximum period of ten weeks, with comparisons being independently carried out on fruit pertaining to weeks: five (N = 20 samples), six (N = 20 samples), seven (N = 20 samples), eight (N = 10 samples), nine (N = 20 samples) and ten (N = 20 samples). The standard that had been established was used to verify whether the samples stored for the remaining weeks (weeks five to ten in cold storage) continued to comply with the quality standard initially established, in other words a quality control test was applied. The data were processed using WinISI II software package ver. 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) to calculate the PCA and the spectral distances based on GH (Shenk and Westerhaus, 1991).

The limits for the Shewhart charts are the extreme percentiles of the in-control distribution of the plotted statistic. When these are means, this is usually assumed to be normal. However, the distribution of GH is non-normal, so in order to calculate the warning limit and action limits for GH, a program was developed in MatLab software (version 2015a, The Mathworks, Inc., Natick, Massachusetts, USA). The GH statistic in WinISI is defined as D/p, where D is the Mahalanobis distance and p is the number of principal component or partial least squares (PLS) factor scores used to calculate D. For data originating from a normal distribution, the distribution of D is χ^2 with p degrees of freedom. This distribution has mean p, so GH=D/p has mean 1. To construct a control chart, the mean line is positioned at level 1, while the upper warning and action limits are positioned at the levels that correspond to the 97.5% and 99.5% percentiles of χ^2_p divided by p. Small GH values are not indicative of problems, so the chart does not require lower limits.

Subsequently, the GH calculated for the various samples stored for between five and ten weeks were represented in the aforementioned chart, with the goal of identifying the orange fruit that did not fulfil the quality standard established by the industry. In addition, the data was used to determine whether the optimal period of cold storage, complying with this standard, could or could not exceed four weeks.

Then, in order to interpret the results of the preceding spectral analysis and employing the reference data for the quality standards i.e. weight, firmness, SSC, TA and BrimA, the Shewhart control charts were created for these parameters. The mean of the parameter and the standard deviation was calculated with the reference data of the 80 samples comprising the standard, as well as warning and action limits, in this case ± 2 and 3 times the standard deviation, assuming a normal distribution for the plotted statistics. These charts displayed the values exhibited by the selected quality parameters for samples kept for between five and ten weeks in cold storage.

In order to explore further a PLS analysis was carried out for each of the firmness and SSC parameters, again creating Shewhart control charts for the GH values from these PLS analyses, using the GH values of the 80 control samples to set limits and then displaying and the GH values exhibited by the samples kept for between five and ten weeks in cold storage, and comparing them to the established standard.

3. Results and discussion

3.1. Definition of the quality standard, determination of the optimal storage time and analysis of conformity

Having defined the quality standard based on the PCA with the samples kept for between one and four weeks in cold storage, established the warning and control limits and plotted the rest of the samples in terms of these axes (Fig. 1), the samples from weeks five to ten that did not meet the standard were identified. Thus, in storage weeks five and six, one sample was found beyond the limit in each ~~of them~~ respectively, three samples exceeded the action limit in week seven, two samples exceeded the action limit in week eight, three samples exceeded the action limit in week nine, and one sample exceeded the action limit in week ten.

Figure 1 shows how, in weeks five and six, samples 91 and 118 were clearly anomalous samples from the outset, in other words, the reason they exceeded the limits was not their postharvest evolution, but rather than from the outset they had exceeded the normal limits for samples of oranges of the type being analysed. Thus, sample 91 has a lower weight (160.70 g) than all the samples of that week when the mean for week five was 244.16 g, while sample 118 had a considerably higher titratable acidity score than the rest of the samples that week, with a citric acid reading of 1.08%, when the mean citric acid score for week six was 0.74% (data not shown).

These results suggest that, although the postharvest duration of oranges kept in cold storage by the citrus industry in the south of Spain has been set at four weeks, this period could be extended by another two weeks, up to six weeks without compromising quality standards. This option would enable the industry to adapt to demand and to fluctuations in prices by prolonging postharvest cold storage for up to two weeks in periods when this would prove advantageous. However, from week seven onwards the samples start to deviate more often from the standard, exceeding the warnings and limits in place.

Subsequently, by employing the evolution of the quality parameters data during cold storage, Shewhart control charts were created in order to better understand which factors have a bearing on the postharvest deterioration of the produce and what is the most limiting parameter or parameters for maintaining postharvest quality during cold storage (Fig. 2 and 3).

Analysis of the control charts shows that in the control chart for firmness the scores of the samples fall progressively over the course of the cold storage between weeks five and ten, and sample 188 in week ten exceeds the lower warning limit. In the SSC control chart, sample 83 in week five, sample 120 in week six, and samples 151, 160 and 162 in week nine exceed the upper warning limit, while sample 104 in week six and sample 136 in week seven exceed the upper action limit.

Analysing the control charts for the physical-chemical parameters (control charts for weight, TA and BrimA not shown) being studied, it is evident that the firmness and SSC parameters are decisive in establishing the evolution of the quality of the oranges during cold storage, which is consistent with Obeland et al. (2008).

For the results, a further PLS analysis was carried out with the spectral data for the firmness and SSC parameters in order to further elucidate a deeper exploration of the results obtained in the PCA.

Both the PLS analysis for firmness (Fig. 4) and the one for SSC (Fig. 5) revealed 31 samples that exceeded the action limit. Samples 85 and 91 (week five), 118 (week six), 128, 129, 131, 134 and 137 (week seven), 141, 144 and 148 (week eight), 163, 164 and 169 (week nine), and 173, 178, 181, 188 and 190 (week ten) all exceeded the established action limit both in the firmness and the SSC PLS analysis, which indicates that these parameters are linked and are determinant in maintaining the established quality standards during the postharvest cold storage of oranges.

Analysis of Figures 4 and 5 shows that the samples of weeks five and six are the ones that best met the established quality standard, given that all the samples complied, except the samples 85, 91 and 118 in weeks five and six, respectively, for both parameters and the sample 97 for the firmness parameter. Moreover, the samples 85 and 97 exhibited two of the highest citric acid scores in week five (0.73 and 0.81% citric acid, respectively) when the mean for that week was 0.68% citric acid. The failure of samples 91 and 118 to comply with the quality standards has already been alluded to. The PLS analysis, like the PCA analysis, confirms that the postharvest cold storage of the oranges could be extended by another two weeks, i.e., six weeks from the time of harvesting, while maintaining the standard established by the industry. It is evident from the Shewhart control chart for the PCA that the samples exhibit less variation in weeks five and six than in the Shewhart control chart based on the PLS analysis of firmness and SSC. This is an indication, revealing that these two factors clearly determine the postharvest cold storage time of oranges, with the firmness parameter being the most determinant of the two in establishing the commercial shelf-life.

4. Conclusions

The results suggest that spectral NIR analysis combined with the Shewhart control charts derived from the spectral information and the physical-chemical analyses carried out constitute a highly useful tool for monitoring oranges during cold storage, and for determining the maximum postharvest period. The data enables cases of non-compliance with the quality standards established by the industry to be detected. The research may be considered as a viability study for fine-tuning a methodology that enables the application of NIR spectroscopy to the monitoring of processes and products and the establishment of quality control tests in the citrus industry, providing it with a highly flexible and innovative quality control strategy consistent with its goals. Future research will need to employ a broader and more varied set of samples enabling the definition of the quality standard to be more universal, thereby ensuring a more robust model for detecting non-compliant fruit.

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Fig. 1. Shewhart control chart based on the GH values derived from the PCA analysis.

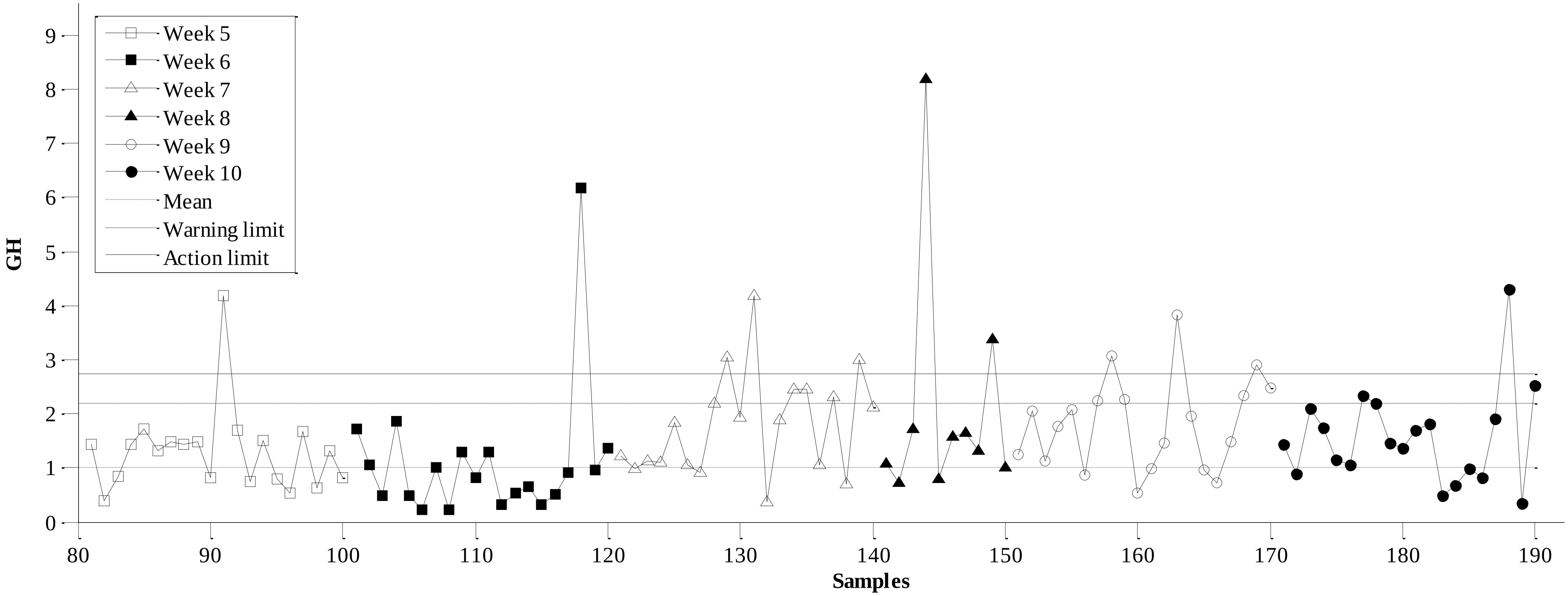

Fig. 2. Shewhart control chart for the firmness parameter.

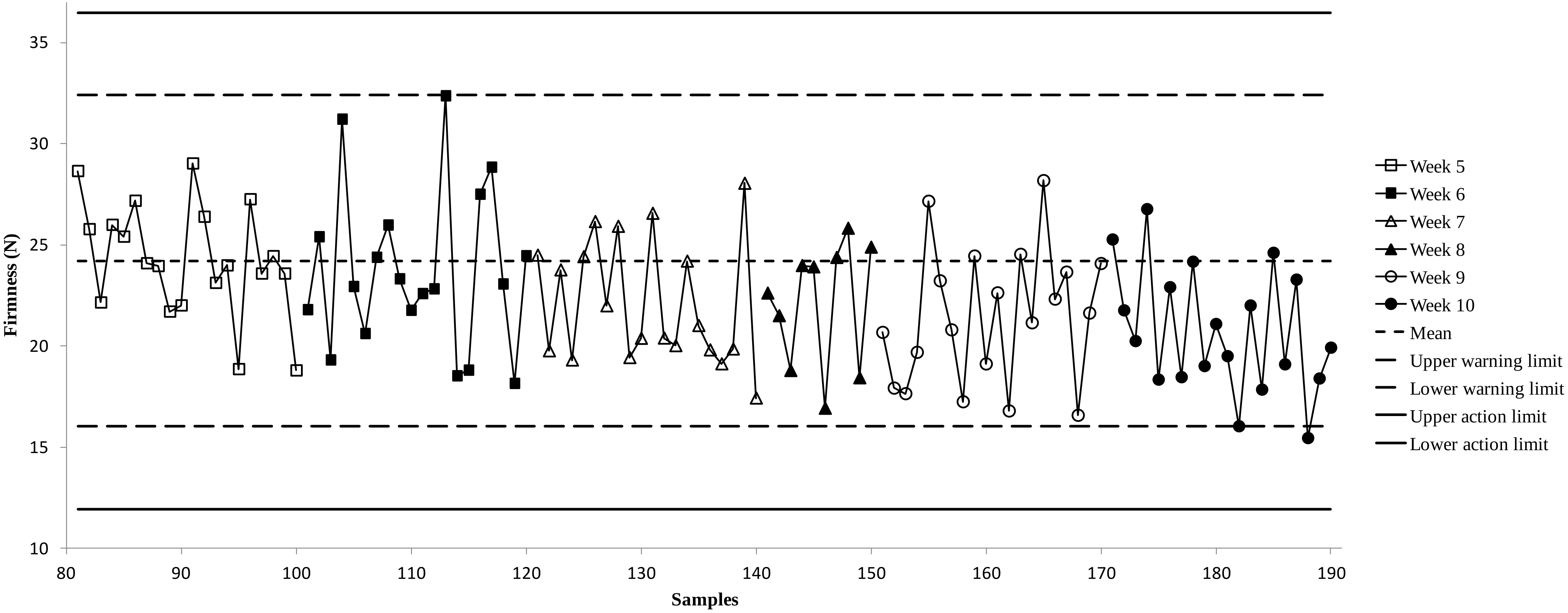

Fig. 3. Shewhart control chart for the SSC parameter.

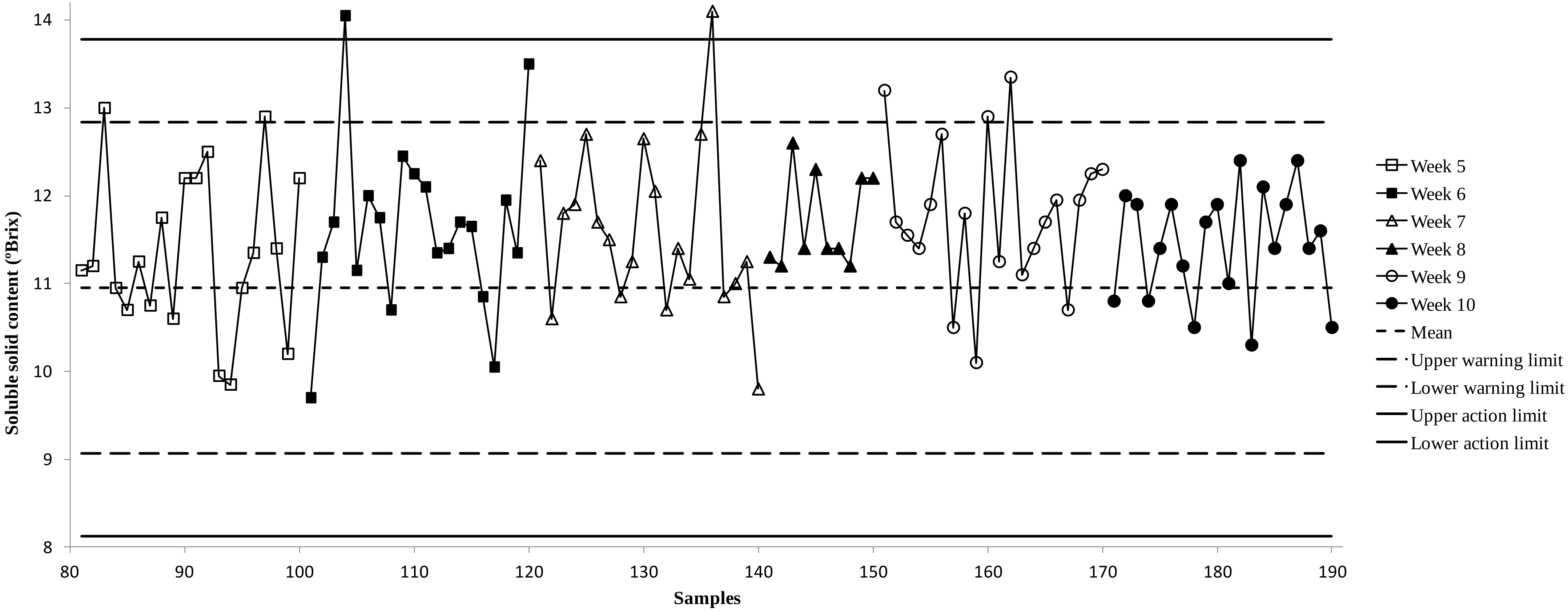

Fig. 4. Shewhart control chart based on the GH values derived from the PLS analysis for the firmness parameter.

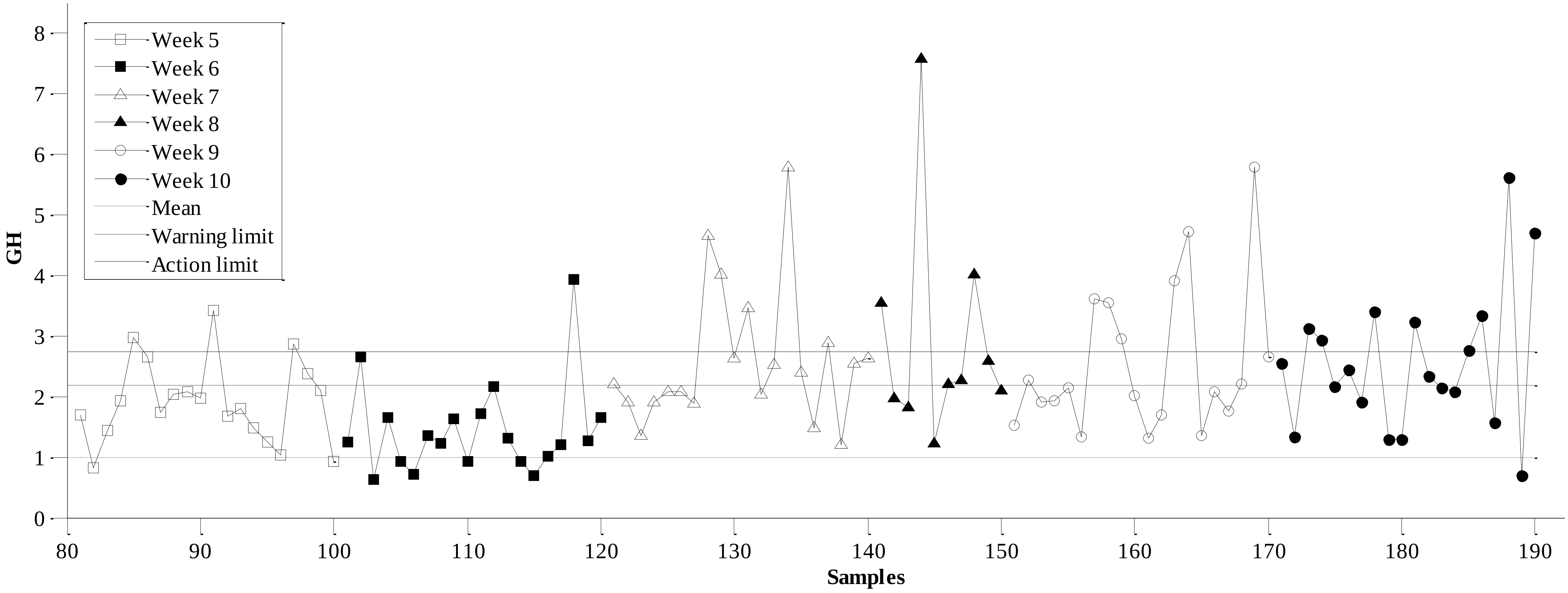

Fig. 5. Shewhart control chart based on the GH values derived from the PLS analysis for the SSC parameter.