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Qualitative Analysis of Apple Fruit during Storage using Magnetic Resonance Imaging

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Abstract

Magnetic resonance imaging (MRI) is a non-destructive technique for determining the quality of fruits which, with different protocols, shows the density and structure of hydrogen atoms in the fruit in which it is placed. This study compared MRI images of healthy and bruised apple flesh tissues, both with and without pests, using various protocols to identify the best one. For this purpose, magnetic resonance imaging (MRI) using two protocols: T_1 (Spin-lattice relaxation time) and T_2 (Spin-spin relaxation time), was carried out on 200 apple fruits that were loaded during storage. The loading of fruits was performed at four levels: 150, 300, 450, and 600 N in a quasi-static manner, and then stored for periods of 25, 50, and 75 days at 4 °C. At the end of each storage period, imaging was carried out. Then, the contrast of T_1 and T_2 images of healthy and bruised tissue of apple fruit with and without pests using ImageJ software was determined. It was concluded that the healthy tissue of apple fruit without pests was clearer in T_1 images than in T_2 images. It has also been seen that the bruised area of fruits without pests in T_2 images is more recognizable than in T_1 images.

Keywords: Apple fruit, Histogram analysis, Magnetic resonance imaging, Quality

Introduction

The apple fruit with the scientific name Malus Domestica is a widely consumed fruit known for its rich content of sugars, vitamins, anthocyanins, minerals, and various other nutrients (Zhang *et al.*, 2019). Nowadays, apples are regarded as one of the most important sources of health benefits due to their components, which include antioxidants, antimicrobial agents, and wound-healing properties (Zhang *et al.*, 2022). One of the factors that caused the drop in the quality of apple fruit is pest. Also, the risk of quality loss is increased by the fact that fresh fruit is highly perishable during storage and transportation (Shicheng, Youwen, Ping, Kuan, & Shiyuan, 2019). Due to the prevalence of pests in apple fruit, as well as compressive forces and external stresses during harvest, transportation, etc., detection of decayed fruit is essential. However, detection of decayed apple fruit mostly relies on manual work. Manual work is inefficient and unreliable (Leiva-Valenzuela & Aguilera, 2013). Therefore, to ensure the minimum acceptability of the fruit's quality to consumers, a healthy, non-destructive, high-accuracy method should be used in the quality sorting of apple fruit.

During the last decade, many researchers have used non-destructive methods to study

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the quality of foods and fruits such as electronic tongue and electronic nose (Lu, Hu, Hu, Li, & Tian, 2022); x-ray computed tomography (Olakanmi, Karunakaran, & Jayas, 2023); ultrasonic wave propagation (Mierzwa, Szadzinska, Gapinski, Radziejewska-Kubzdela, & Biegańska-Marecik, 2022); hyperspectral imaging (Khodabakhshian & Emadi, 2017; Wieme *et al.*, 2022); nuclear magnetic resonance (Ozel & Oztop, 2021; Perez-Palacios *et al.*, 2023); near infrared spectroscopy (Lan *et al.*, 2022) and Raman spectroscopy (Khodabakhshian, 2022). Among these methods, magnetic resonance imaging (MRI) has become well known due to its advantages, such as reliable online quality assessment, excellent soft tissue differentiation compared to X-ray imaging, and its applications in studying the ripening stage and ripening of fruits, pathogen invasion, tissue chemistry, water transfer and diffusion, and oxygen diffusion in agricultural products (Srivastava, Talluri, Beebi & Kumar, 2018; Perez-Palacios *et al.*, 2023). On the other hand, this non-destructive technique can analyze the distribution and motility of protons in water molecules and other metabolites concentrated in biological tissue. So, it is usable to determine the change in the concentration of oil and water in food and agricultural products, which is usually associated with maturity, damage and fruit rot (Perez-Palacios *et al.*, 2023).

Usually, three types of protocols are used in magnetic resonance imaging: T_1, T_2, and proton density-weighted images. T_1 and T_2 are two completely separate time parameters that can be detected and measured after RF pulse excitation. The comparison between these two times and their ratio in different states is the basis of magnetic resonance imaging (Perez-Palacios *et al.*, 2023). The MRI imaging protocol is determined by considering the structure of the tissue being imaged in terms of the density of water and hydrocarbon molecules.

Many researchers have used magnetic resonance imaging to study agricultural products (Defraeye *et al.*, 2013; Galed, Fernández-Valle, Martinez, & Heras, 2004; Gonzalez *et al.*, 2001; Herremans *et al.*, 2014; Mazhar *et al.*, 2015; Ozel & Oztop, 2021; Perez-Palacios *et al.*, 2023; Razavi, Asghari, Azadbakh, & Shamsabadi, 2018; Shicheng *et al.*, 2019; Zhang & McCarthy, 2012). In one research, the effect of force and storage time on the distribution of bruising volume of Darghazi pears was studied with the help of image data obtained from magnetic resonance imaging, and they concluded that the applied force leads to a linear increase in the bruising volume during the storage period, while the effect of storage time on the diffusion of distribution of bruising is non-linear (Razavi *et al.*, 2018). Shicheng *et al.* (2019) used low-field nuclear magnetic resonance (LF-NMR) data to detect decayed blueberry fruit from healthy. Also, in another experiment, Gonzalez *et al.* (2001) investigated the development of internal tissue browning due to high levels of carbon dioxide in the storage of Fuji apples in a controlled atmosphere, and it was concluded that T_2 measurements of images produced better contrast between normal tissue and tissue with intrinsic browning compared to the image produced using differences in proton density or T_1 measurements.

Therefore, the aim of the current research was to determine which MRI protocol performed best for different parts of the apple fruit. The two imaging protocols, T_1 and T_2, were compared across various areas of the apple, including the healthy tissue, bruised areas, and the fruit core, using the desired protocols for both pest-free and pest-infested apples.

Materials and Methods

Sample collection and preparation

In this experiment, a total of 200 apple fruits of the same size, grown in 2021 from a commercial orchard in Mashhad, Khorasan Razavi province, Iran were randomly collected. The physico-chemical properties of the studied apple variety are shown in Table 1. The fruits were divided into two groups, based on the subjective evaluation of their skin texture: (i) Healthy fruits without pests, and (ii) Infected fruits infested with pests. Then all samples were individually washed, numbered and placed in plastic boxes. After selection, apples were transported to the physical properties laboratory and stored in periods of 25, 50, and 75 days at 4 °C. Before starting the experiment, the samples were taken out of the refrigerator and placed at room temperature (22°C) for approximately 2 hours in order to reach temperature equilibrium (Khodabakhshian *et al.*, 2017). MRI experiments were performed on all samples.

Table 1- Physico-chemical properties of the studied apple variety

Variety	Soluble Solids (Brix)	Titratable Acidity (gL^{-1})	pH	Geometric mean diameter (mm)	Fruit density (gcm^{-3})	Moisture (% w.b.)
Golden Delicious	13.2 ± 0.4	0.6 ± 0.1	3.5 ± 0.2	65.09 ± 5.2	0.94 ± 0.12	86.68 ± 0.13

In order to study apple susceptibility to bruising during the storage period, the quasi–static compression was used to create the bruised area. The quasi– static compression force was exerted on equatorial regions of samples of each group by the same probe (plunger) using Mechanical Testing Machine (Model H5KS, Tinius Olsen Company) with a load cell of 5 4903.33 N. Each individual sample was loaded at pretest speed 1.5 mm min^{-1}, the test speed of 0.5 mm min^{-1}, four levels 150, 300, 450, and 600 N (Khodabakhshian, Emadi, Khojastehpour, & Golzarian, 2019). These four levels were selected to load the samples (in three replications) according to the initial tests on the fruit. It was observed that a force higher than this amount caused the complete failure of the fruit and on the other hand, a force less than about 150 Newtons did not have a significant effect on the fruit. In this experiment, samples were positioned horizontally (Figure 1) during loading, and the amount of force-deformation of the fruits were recorded.

MRI measurements

Magnetic resonance imaging was performed with two protocols T_1 and T_2 to examine the differences between these types of imaging and to detect healthy, infected, and bruised areas of the samples, as well as the ability to detect its various components and tissues. T_1 imaging differentiates between adipose tissue and water, showing water as lighter than adipose tissue. T_2 imaging, similar to T_1-weighted imaging, separates fat and water but with the difference that fat appears lighter and water appears darker in the image (McRobbie, Moore, Graves, & Prince 2009; Perez-Palacios *et al.*, 2023). As it was stated in section 2.1, the samples were stored for periods of 25, 50, and 75 days at 4 °C in a refrigerator. At the end of each period of storage, magnetic resonance imaging with T_1 and T_2 protocols was performed using Alltech EchoStar 1.5T magnetic resonance imaging device in Aref Imaging Medical Center in Mashhad, Khorasan Razavi province, Iran (Figure 2). These images were acquired with a field of view of 350 × 350 mm, thickness of 3 mm, pixel depth of 3 mm, recovery time (TR) of 905 ms, effective echo time (TE) of 10 ms for T_1, and TR of 5598 ms and TE f 100 ms for T_2. The total acquisition time was 4 min 2s for all the slices, for all experiments (Herremans *et al.*, 2014; Noshad, Asghari, Azadbakht, & Ghasemnezhad, 2020). For each fruit, approximately 45 slices were obtained, ranging from 43 to 47, depending on the apple size. ImageJ software was used to compare the contrast in T_1 and T_2 images. With help of this software, samples of healthy tissue, infected tissue, and bruised tissue due to quasi-static compression were studied and the histogram of these samples were compared.

Data Analysis

A completely randomized design (CRD) in factorial with two experimental factors was employed, studying two factors: loading force and storage period. These two factors were tested across both healthy and infected fruit groups, with three replications. All data were subjected to one-way analysis of variance, ANOVA using SPSS19 software. The F test was used to determine the significance of independent factors (loading force and storage period), and significant differences of means were compared using the Duncan's multiple ranges test at 5% significant level.

Results and Discussion

The results of variance analysis which was carried out to examine the effect of loading variables, storage time and their interaction on the amount of light intensity in images taken with protocols T_1 and T_2 for flesh tissue and bruised part of apple fruit without pests and with pests is shown in Table 2.

Table 2- Analysis of variance of factors considered on the T_1 and T_2 images of apple fruit

Factors	df	T_1 image of flesh tissue without pests	T_2 image of flesh tissue without pests	T_1 image of bruised tissue without pests	T_2 image of bruised tissue without pests
		Mean squares			
Loading force	3	1597.72*	2896.51**	88.91^{ns}	205.11^{ns}
Storage period	2	1884.51**	314.23^{ns}	8652.13**	3096.22**
Loading force × Storage period	6	625.23*	481.22^{ns}	65.11^{ns}	29.53^{ns}
Error		118.12	156.62	97.15	42.84
Coefficient of variation		10.25	12.26	19.42	20.72
	df	T_1 image of bruised tissue with pests	T_2 image bruised tissue with pests	T_1 image of flesh tissue with pests	T_2 image flesh tissue with pests
Loading force	3	682.42**	47.62*	432.86^{ns}	1976.85*
Storage period	2	9586.22**	3982.27**	38.56^{ns}	2453.22**
Loading force × Storage period	6	212.91**	38.52^{ns}	796.22^{ns}	352.91^{ns}
Error		31.25	22.47	683.57	501.22
Coefficient of variation		15.76	18.74	19.49	22.05

** Significant at the 1% level, * Significance at the 5% level, and ^{ns} Non – significant

Histogram analysis of T_1 and T_2 images of flesh tissue of apple fruits without pests

A sample of images with T_1 and T_2 protocols related to flesh tissue of pest-free apple fruits without applying force and also the histogram of a sample of their selected area are shown in Figure 1. As can be seen in this figure, the intensity of brightness in the T_1 images is higher than in the T_2 images. Also, the standard deviation of the sample histogram with T_2 protocol is more than T_1 protocol, which indicates more fluctuations in images with T_2 protocol. Therefore, it can be concluded that the weighted images taken with protocol T_1 are completely different. That is, tissues with a long T_1 have the weakest signal, which causes the T_1 images to be brighter. In other words, the bright pixels in the images with the T_1 protocol are related to short T_1 (1500-2000 ms) (Li, Li, & Zhang, 2018; Shicheng *et al.*, 2019).

According to the results obtained for flesh tissue of apple fruits without pests with T_1 protocol, loading force ($p<0.05$), storage period ($p<0.01$), and their interaction ($p<0.05$) were significant (Figure 2a). Additionally, on the basis of the acquired results, with increasing storage time and loading force, the brightness (from 0 to 255) of flesh tissue of pest-free apple fruits decreased with T_1 protocol and its value became darker (got closer to zero), which means a change in tissue color due to pressure. No statistically significant differences were observed in the loading forces during the storage periods of 25 and 50 days, nor in the forces of 450 and 600 N across the storage durations of 1, 25, 50, and 75 days. Also, as can be found from Table 2, only the loading force was significant ($p<0.01$) and the storage period and their interaction were not significant for flesh tissue of apple fruits without pests with T_2 protocol. According to Figure 2b, it is apparent that there is a significant difference between all four loading forces and the brightness significantly decreased with increasing of loading force so the maximum and minimum of brightness belonged to loading forces of 150 N and 600 N, respectively.

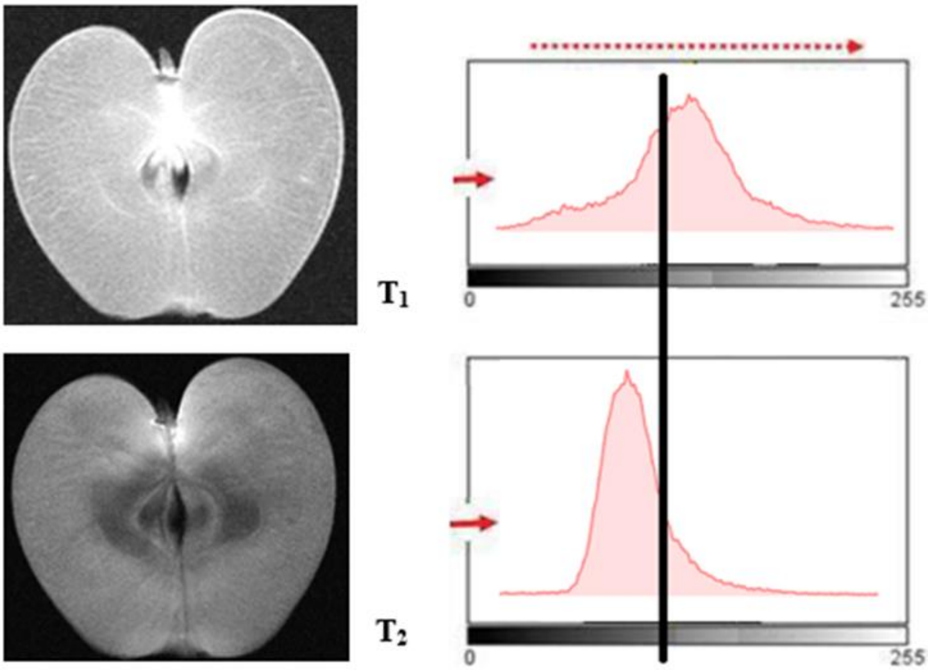

Fig. 1. Histogram of magnetic resonance imaging with T_1 and T_2 protocol of pest-free apple fruits without applying force

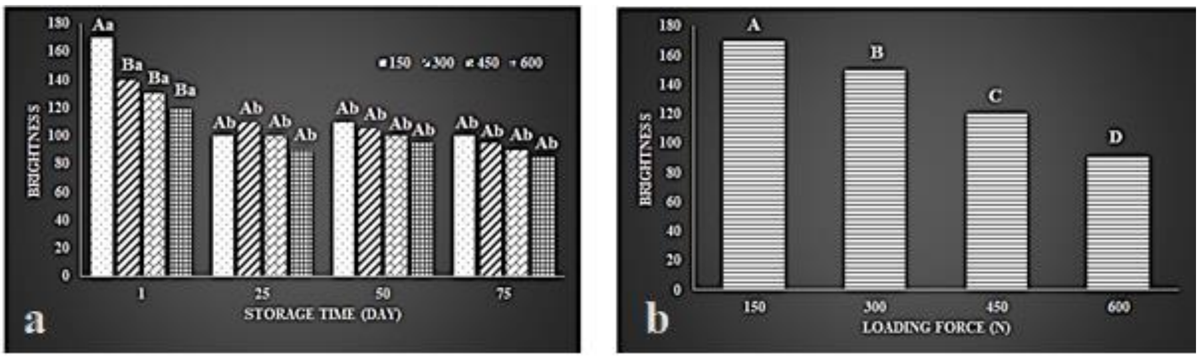

Fig. 2. **a:** Interaction effect of loading force and storage time on light intensity of flesh tissue of apple fruits without pests with T_1 protocol. **b:** Comparison of the mean effect of loading force on the brightness of flesh tissue of apple fruits without pests with T_2 protocol. Uppercase letters indicate insignificance in a fixed storage period and lowercase letters indicate insignificance in a fixed loading force

Histogram analysis of T_1 and T_2 images of flesh tissue of apple fruits with pests

Figure 3 shows an example of images with T_1 and T_2 protocols related to flesh tissue of apple fruits with pests. As it can be seen, the track of the pest passage in T_1 and T_2 images is quite clear, although due to the presence of hydrocarbon residues of the pest, fewer details of the track were detectable in T_1 images. At first, the pupae of the "Apple" fruit pest enter the "Apple" seeds, and since the "Apple" seeds are seen in T_1 images, the details of the pest infestation of the seeds are more specific in this type of imaging. Similarly, Haishi, Koizumi, Arai, Koizumi, and Kano (2011) investigated the contamination of apple fruits harvested by peach fruit moth (*Carposina sasakii Matsumura*) using non-destructive magnetic resonance imaging (MRI) and T_1 and T_2 protocols. Similar researches were also done by Herremans *et al.* (2014), Mazhar *et al.* (2015), Shicheng *et al.* (2019), and Noshad *et al.* (2020) on apple, avocado, blueberry, and quince fruits, respectively, using low field nuclear magnetic resonance and T_1 and T_2 images.

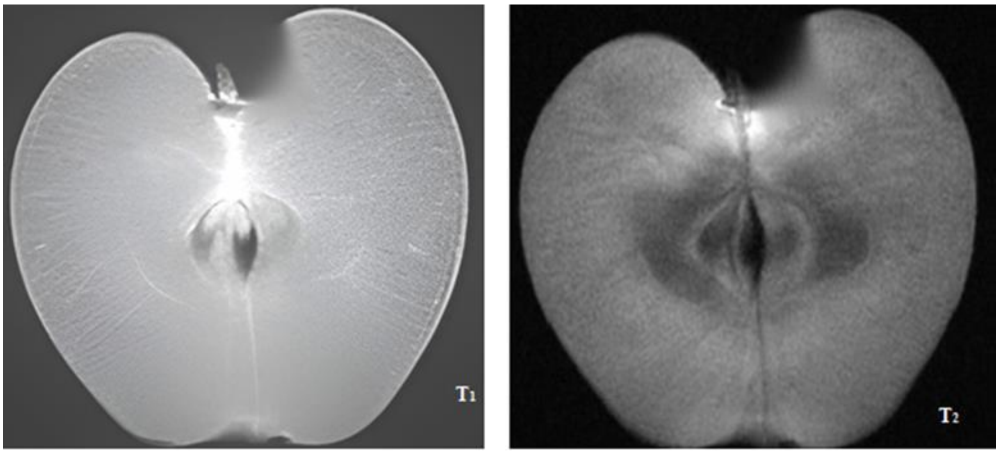

Fig. 3. Comparison of magnetic resonance images of T_1 and T_2 protocols related to flesh tissue of apple fruits with pests

According to the results (Table 2), none of the factors were significant for the flesh tissue of infected apple fruits with T_1 protocol, but with T_2 protocol, the effect of two factors of loading force and storage period were significant ($p<0.01$). As it can be found from Figure 4, the light intensity of flesh tissue of infected apple fruits "goes" to the blurred, of course, there was no statistically significant difference between 300, 450, and 600 N forces, but these three forces had a significant difference with the force of 150 N. It can also be seen that with increasing storage period, the light intensity of flesh tissue of pest infested apple fruits become darker and of course there is no statistically significant difference between samples of storage period after 25, 50, and 75 days. However, there are statistically significant differences between these three storage periods and the samples taken on the first day.

Histogram analysis of T_1 and T_2 images of bruised tissue of apple fruits without pests

As shown in Figure 5, the bruised area is clearer in the T_2 protocol image. This is due to the exit of more water molecules and the decrease of moisture from the cells of this area and absorption by other areas during 75 days of storage, but in the image with the T_1 protocol, there is not much difference in the area bruised compared to other areas because the loaded areas show lower water content (shorter T_2 time) compared to unloaded areas with higher water content (longer T_2 time). This justification was also presented by McRobbie *et al.* (2009).

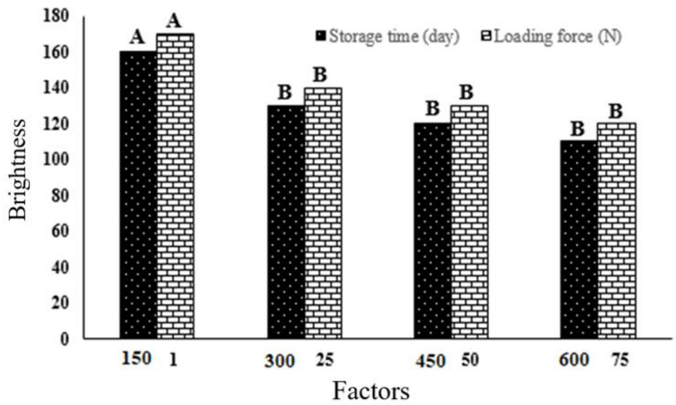

Fig. 4. Mean Comparison of the effect of loading force and storage period on the brightness of the T_2 images of flesh tissue of pest infested apple fruits

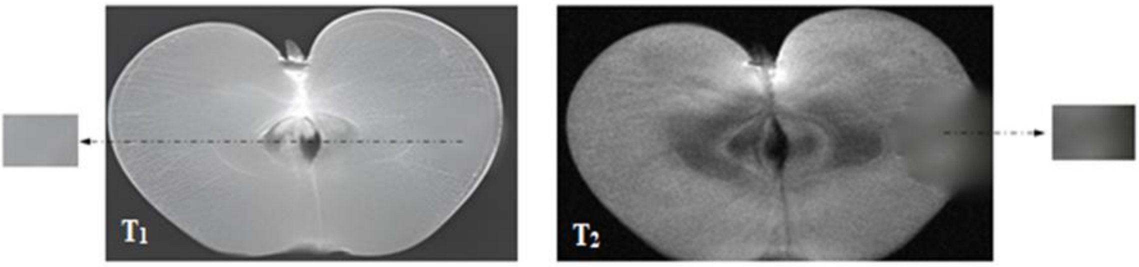

Fig. 5. Difference between T_1 and T_2 magnetic resonance images of the bruised tissue of apple fruits without pests after 75 days of storage

For the bruised tissue of apple fruit without pest with T_1 protocol only the storage period was significant ($p<0.01$) and their loading force and interaction were not significant (Table 2). The results of comparing the mean in Figure 6 show that as the storage period increases, the brightness of the bruised fruit with the T_1 protocol becomes darker and there is a statistically significant difference between them. Only the storage period was significant ($p<0.01$) for the pest-free bruised tissue of apple fruit with T_2 protocol and the loading force and the interaction of these two factors were not significant. According to Figure 8, with increasing storage period, the amount of darkness for the bruised tissue increases and a significant difference is observed between the two storage periods of 50 and 75 days. In a similar experiment on Fuji apples, Gonzalez *et al.* (2001) studied the development of internal tissue bruising due to high levels of carbon dioxide in controlled atmospheric storage and concluded that T_2 measurements of images with better contrast provide a contrast between normal tissue and tissue with internal browning relative to the image produced using differences in proton density or T_1 measurements. These results were also similar to the results of Hernández-Sánchez, Hills, Barreiro, & Marigheto (2007), Defraeye *et al.* (2013), and Noshad *et al.* (2020) on internal browning of pear, apple, and quince fruits using T_2 protocol, respectively.

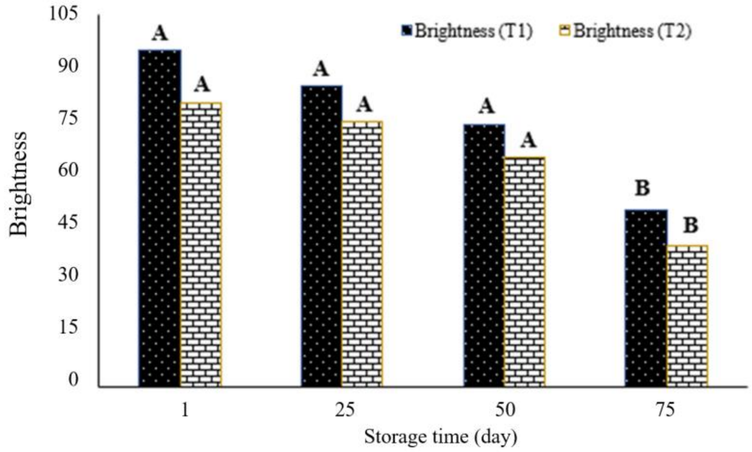

Fig. 6. Mean Comparison of the effect of storage period on the brightness of T_1 and T_2 images of bruised tissue of apple fruit without pest

Histogram analysis of T_1 and T_2 images of bruised tissue of apple fruits with pests

Figure 7 shows the difference between the magnetic resonance images with the T_1 and T_2 protocols of bruised tissue of apple fruits with pests. As shown in the figure, the bruised tissue with the T_1 protocol is clearer, because the infected tissue loses its moisture by creating an empty space by the pest over time. Also, the loaded area is darkened due to the decrease in moisture because with increasing interval the humidity decreases after loading (Diels *et al.*, 2017; Noshad *et al.* 2020). According to the results (Table 2), the contaminated tissue of apple fruit bruised with T_1 protocol was significant for both loading force and storage period ($p<0.01$) and the interaction of these two factors was also significant. Furthermore, based on Figure 8, it can be concluded that the light intensity of the bruised tissue of apple fruit decreased with increasing storage period and this was observed for all four loading forces.

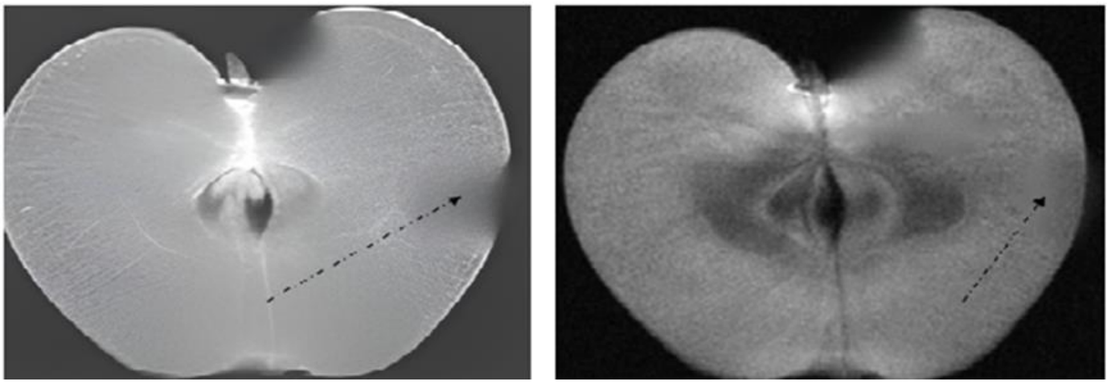

Fig. 7. Difference between T_1 and T_2 magnetic resonance images of the bruised area of apple fruit with pest

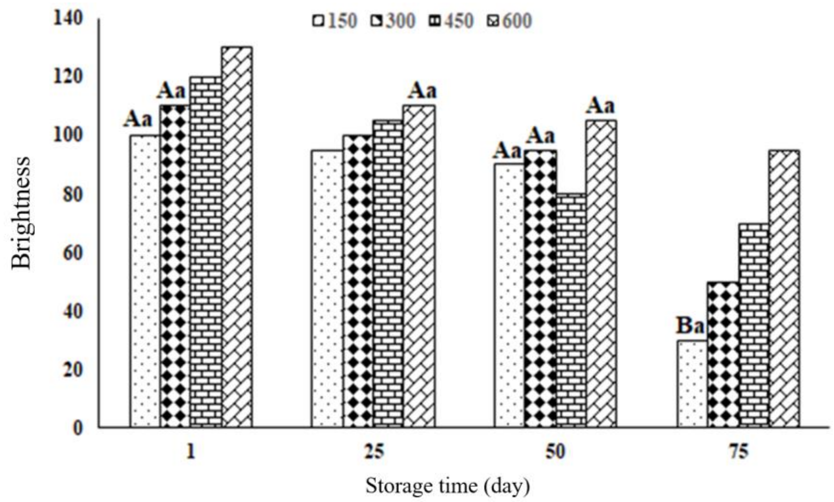

Fig. 8. Interaction effect of loading force and storage time on light intensity of bruised tissue of apple fruits with pests by T_1 protocol. Uppercase letters indicate insignificance in a fixed storage period and lowercase letters indicate insignificance in a fixed loading force

There was no statistically significant difference between loading forces for samples in the storage period of 25-day and also there was no statistically significant difference between loaded forces of 150 and 600 N for 50-day storage period, but a significant difference was observed between all four loading forces in 75-day storage period. In addition, the effect of loading force parameters ($p<0.05$) and storage period ($p<0.01$) on the bruised tissue of apple fruits with pests by T_2 protocol were significant and the interaction of these two factors was not significant. According to Figure 9, it is clear that with increasing loading force, the amount of bruised tissue in the infected fruit became darker and a statistically significant difference was observed between the forces of 150 and 600 N for the specified value. Also, with increasing the storage period from 25 to 75 days, the light intensity of the bruised fruit tissue becomes darker and there is a statistically significant difference between these storage periods. These results were also similar to the results of Noshad *et al.* (2020) on internal browning of quince fruit using low field nuclear magnetic resonance and T_1 and T_2 images.

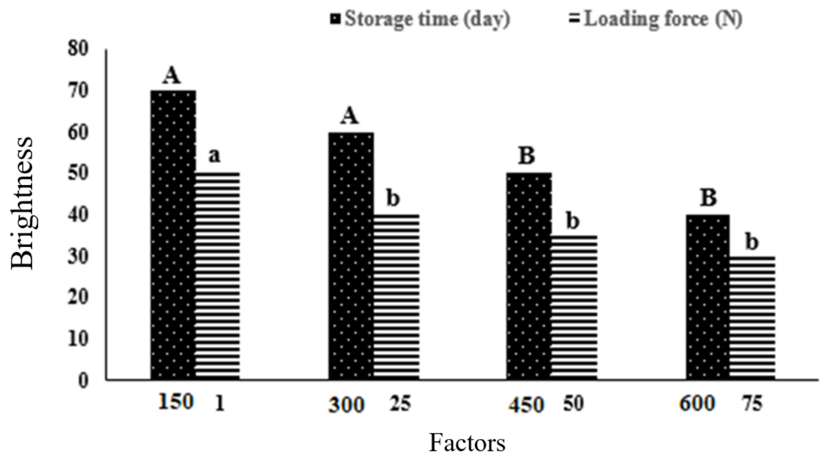

Fig. 9. Mean Comparison of the effect of storage period and loading force on the brightness of T_1 images of bruised tissue of apple fruit with pest

Conclusion

This study investigates the application of magnetic resonance imaging (MRI) for qualitative analysis of apple fruit during storage, focusing on both healthy and pest-infected samples. The research employs T_1 (spin-lattice relaxation time) and T_2 (spin-spin relaxation time) MRI protocols to assess differences in tissue structure and water content over a storage period of 25, 50, and 75 days at 4°C. A total of 200 apples were subjected to quasi-static loading at four levels (150, 300, 450, and 600 N) to induce bruising, mimicking conditions encountered during handling and transportation.

The key findings indicate that T_1 imaging provides clearer differentiation of flesh tissue in pest-free apples, whereas T_2 imaging enhances the visibility of bruised areas, particularly in apples without pest. In the case of pest-infected apple fruits, both T_1 and T_2 MRI protocols revealed distinct characteristics of tissue integrity and pest infiltration. T_1 imaging showed identifiable tracks of pest pathways, particularly around seeds, where the infestation was more pronounced. Meanwhile, T_2 imaging provided clearer visualization. The study highlights significant effects of loading force and storage duration on MRI image contrast, revealing distinct patterns in tissue degradation and water distribution. Histogram analyses of T_1 and T_2 images further illustrate these differences, showing variations in brightness and standard deviation across different experimental conditions.

Overall, MRI proves to be effective in non-destructively assessing the quality of apple fruit, offering insights into structural changes, moisture loss, and pest-induced damage. This research underscores the potential of MRI as a robust tool for quality assessment in agricultural produce, contributing valuable data to improve storage and transportation practices.

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Declaration of competing interests

The authors declare that they have no conflict of interest.

Authors Contribution

R. Khodabakhshian Kargar: Supervision and management, Data collection, Data processing, Statistical analysis, Validation, Extracting, and preparing the primary text

R. Baghbani: Conceptualization, Methodology, Technical consultation, Software services, Interpreting the results, Editing, and translating the text.

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تجزیه و تحلیل کیفی میوه سیب در طول ذخیره‌سازی با استفاده از تصویربرداری تشدید مغناطیسی

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چکیده

تصویربرداری تشدید مغناطیسی (MRI)، یک روش غیرمخرب برای تعیین کیفیت میوه‌ها است که با پروتکل‌های مختلف، چگالی و ساختار اتم‌های هیدروژن را که در آن قرار می‌گیرد نشان‌می دهد. در این مطالعه تصاویر MRI گرفته‌شده با پروتکل‌های مختلف از بافت گوشتی و قسمت کبودشده میوه سیب بدون آفت و با آفت مقایسه و بهترین پروتکل معرفی شد. برای این منظور، تصویربرداری تشدید مغناطیسی (MRI) با استفاده از دو پروتکل T_1 و T_2 بر روی ۲۰۰ میوه سیب بارگذاری‌شده در حین نگهداری انجام شد. بارگیری میوه‌ها در چهار سطح ۱۵۰، ۳۰۰، ۴۵۰ و ۶۰۰ نیوتن به‌صورت شبه‌استاتیک انجام شد و سپس در دوره‌های ۲۵، ۵۰ و ۷۵ روزه در دمای ۴ درجه سانتی‌گراد نگهداری شد. در پایان هر دوره ذخیره‌سازی، تصویربرداری انجام شد. سپس کنتراست تصاویر T_1 و T_2 صدا و بافت کبودشده میوه سیب با و بدون آفت با استفاده از نرم‌افزار ImageJ تعیین شد. نتیجه‌گیری شد که بافت صوتی میوه سیب بدون آفت در تصاویر T_1 واضح‌تر از تصاویر T_2 بود. همچنین دیده شده است که ناحیه کبودی میوه‌های بدون آفت در تصاویر T_2 بیشتر از تصاویر T_1 قابل‌تشخیص است.

واژه‌های کلیدی: آنالیز هیستوگرام، تصویربرداری تشدید مغناطیسی، کیفیت، میوه سیب

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