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Freezing, thawing and cooking effects on quality profile assessment of green beans (cv. *Win*)

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Abstract Results are presented of the effect of freezing followed by thawing (air and water immersion, both at environmental temperature) and cooking (traditional boiling in a covered pot) on quality **profile (in terms of objective texture, colour, chlorophylls and pheophytins and sensory attributes)** and structure of green beans (cv. *Win*). Freezing was carried out at three different rates by forced convection with liquid nitrogen vapour. Kramer shear cell (KSC) and Warner-Bratzler (WB) tests were used for objective assessment of the texture. The highest parameter values occurred in beans frozen at the highest rate and air-thawed at the slowest rate. Also, minimum alteration of the rheological behaviour of cooked beans was achieved by freezing at the highest rate. The best parameter for assessing the texture of frozen green beans after thawing and cooking was the Warner-Bratzler slope (S_{WB}). Coefficients of softening estimated for S_{WB} in the thawed beans showed that the texture of the beans frozen at $-24\text{ }^{\circ}\text{C}$ was almost 4 and almost 5 times softer than that of the beans frozen at $-70\text{ }^{\circ}\text{C}$, for air and water thawing respectively. Frozen and thawed green beans were darker than fresh control, whereas freezing prior to cooking produced lighter-coloured beans than direct cooking. The freezing rate affected colour parameters differently depending on the process that followed. When beans were thawed, increasing the freezing rate produced lighter-coloured beans, whereas when beans were cooked, increasing the rate produced darker-coloured beans. No difference was found in sensory assessments between cooked samples frozen at -24 , -35 and $-70\text{ }^{\circ}\text{C}$, which probably reflects the panellists' mixed preferences for quickly and slowly frozen samples. **Scanning electron microscopy (SEM)** revealed different degrees of mechanical damage to tissue structure, which accounted for the rheological behaviour of the beans.

Keywords *Phaseolus vulgaris* L. · Freezing and thawing rates · Cooking · Mechanical tests · **Quality profile · Sensory analysis · Structure**

Introduction

Green beans (*Phaseolus vulgaris*, L.) are one of the most important vegetables produced in Portugal, where they are much appreciated for their high quality, both in nutritional (e.g. vitamins, fiber and starch) and sensory properties (e.g. colour, texture and flavour). Green beans are mostly grown outdoors and harvested seasonally, which makes freezing **very important process** **preservation for extending their availability throughout the year** [1]. **However**, the market for ready meals in Europe expanded rapidly in the period 1995-1999, with growth underpinned by beneficial macroeconomic conditions and increasing demand for convenience foods [2]. Frozen ready meals are the largest sector in terms of volume and value, and there is an increasingly wide variety of frozen ready meals available. A whole range of frozen vegetable-based ready meals has been launched by the industry in the last few years in Spain [3]. Marketing is focused on convenience, as these products require only 5-10 **min** preparation.

The beneficial effects of rapid freezing rates on structure and texture are reflected by the results of various methods of texture analysis (i.e. histological, sensory imitative and objective techniques) in studies by Gutschmidt, Brown and Canet on green beans [4, 5, 6]. Using objective methods of texture analysis, Brown [7] successfully detected the positive effect of rapid freezing rates, even after cooking, in green beans. More recently, Chourot et al. [8] have shown that frozen beans were frozen up to four times faster by immersion freezing than by air-blast freezing, and sensory panellists preferred the former because of their texture. Vegetable colour is also an important sensory property and may be used as a criterion of vegetable quality [9]. For this reason, other studies have focused on the effects of freezing on the pigment content (mainly chlorophylls)

in green beans, which are a major source of dietary magnesium [10]. The predominant chlorophylls in green beans are chlorophylls *a* and *b*, which generally occur at a ratio of about 3:1 [11].

There are several different stages in the production of frozen vegetables during which significant loss of product quality can occur: initial processing (blanching) and preparation prior to freezing; freezing; frozen storage following freezing; and above all, final cooking prior to consumption [3, 12]. For example, it is known that the main loss of nutrients in frozen vegetables typically occurs during blanching, a high temperature process which inactivates enzymes that cause deterioration in colour, flavour and odour [6, 13, 14]. Also, recent studies have shown that blanching was accompanied by a decrease in green bean firmness at 85, 90 and 97 °C following simple first-order kinetic models [15]. Once it was accepted that vegetable freezing was influenced by other stages of the thermal treatment (such as blanching and cooking), and other factors such as product type, packaging, and storage time, the freezing rate ceased to be considered the prime factor affecting final quality [6]. However, this is not meant to suggest that the freezing rate does not in fact affect quality, since it is true that most fruits and vegetables lose quality when frozen slowly.

The texture and the consumer quality of frozen green beans may ultimately be determined in the final stage of the overall process: that is the way they are cooked. For example, beans lose less pigmentation when boiled in a covered pot, pressure cooked or microwaved than when steamed. Pressure cooking also generally entails little loss of nutritious components and is therefore recommended in preference to the traditional method of boiling in a covered pot [16]. However, the study was conducted on fresh green beans, and so the results may not be applicable to previously processed beans. Thawing of frozen green beans is another operation that significantly

compromises quality. Thawing properly is essential to maximise quality retention and safety of frozen vegetables [17]. Thawing inside a refrigerator at temperatures below 5 °C or as a part of the cooking process, are considered microbiologically safe procedures. Simple direct cooking is recommended as part of any meal preparation, since thermal processing assures microbiological safety as compared to thawing inside a refrigerator or at ambient temperatures. In contrast, thawing inside a refrigerator or at ambient temperature is important during preparation stages (e.g. for salads and pies) and where processing and/or loss of soluble solids by water immersion thawing are not desired. And thawing may ultimately nullify the effect of controlling temperature throughout the frozen foods distribution chain, since quality can be severely affected at this final stage [17]. As noted earlier, retention of quality in frozen beans is also influenced by packaging materials, and computational models have shown that a good insulating material can significantly reduce the effect of temperature abuses by increasing thawing times by up to 190% [1].

Those processes that can contribute to loss of final quality of frozen vegetables therefore need to be studied separately; however, it must be borne in mind that there are significant interactions between them. The objective of this work was to determine how freezing rate, either after thawing at ambient temperature in two different media (with a view to producing frozen beans for salads and pies) or after cooking (recommended as part of any meal preparation) affects the quality and structure of *Win* green beans. A secondary aim was to determine what rheological parameters will best account for the mechanical behaviour of the tissue in response to structural damage caused by a variety of freezing conditions.

Materials and methods

Test material

The raw material, supplied by the industry (Carreira, Portugal), consisted of 70 kg of fresh green beans (*Phaseolus vulgaris* L.) of the variety cv. *Win*, which is round. The fresh material was kept at 4 °C and 90-95 % relative humidity throughout the experiment. In each experimental unit (sample subjected to each different freezing and thawing or cooking combination), undamaged pods at the optimum stage of maturity were gauged and only pods having a diameter ranging from 9.5 to 11 mm (medium calibre) were selected for use in this study [18]. Green bean pods were hand washed after removal of the tips and cut into pieces 4 cm long.

Freezing, thawing and cooking processes

The experimental units were frozen by forced convection with liquid nitrogen vapour in an Air Liquide CCF 40 cabinet until their thermal centres reached -18 °C. Three different freezing rates were assayed: at -24 °C ($R_f = 1.5 \pm 0.05$ °C min⁻¹, the slowest freezing rate), -35 °C ($R_f = 2.7 \pm 0.10$ °C min⁻¹, an intermediate freezing rate), and -70 °C ($R_f = 5.4 \pm 0.10$ °C min⁻¹, the fastest freezing rate). Freezing rates were calculated to be the difference between initial temperature and final temperature divided by freezing time, freezing time being defined as the time elapsing from the start of the pre-freezing stage until the final temperature (-18 °C) has been attained [19]. After freezing, the samples were packed into stratified polyethylene bags (500 g/bag) and sealed under slight vacuum (-0.05 MPa) in a Multivac packing machine to prevent oxidation and surface dehydration damage, and then stored at -22 °C until thawing or cooking. Green beans were thawed at two different rates, slowly by air thawing at ambient temperature (+20 ± 2 °C) ($R_t = 0.35 \pm 0.05$ °C min⁻¹), and quickly by water immersion thawing at ambient temperature (+20 ± 1 °C) ($R_t = 8.40 \pm 0.15$ °C min⁻¹), until their thermal centres reached ambient temperature. Thawing rates were calculated to be the difference between initial temperature (-22 °C) and final temperature (+20 °C) divided by thawing time, thawing time being defined as the time elapsing from the start of the thawing until the ambient temperature has been attained. Since frozen vegetables are traditionally designed to be cooked and then cooled to a temperature suitable for consumption, frozen beans were cooked by traditional boiling procedure [20]. In this case, 400 ml of water with 2 g of salt was brought to the boil; 500 g of beans was then added and the beans were boiled for a further 15 min. For

calculation of the freezing and thawing rates in the different modes [19], media and green beans temperatures were monitored by T-type thermocouples using a hardware and software system (Campbell Scientific, PS 40) that permitted data gathering and storage. The freezing and thawing rates given are the mean values of four different processes conducted under the same conditions.

Instrumental texture measurements

For objective assessment of texture, tests simulating chewing with a Kramer Shear Cell (KSC), and empirical Warner-Bratzler tests were performed, using a Lloyd Instruments mod T5K texturometer (J.J. Lloyd Instruments Ltd., Southampton, UK) [21]. KSC tests were performed with a 5000 N-load cell, whereas Warner-Bratzler tests were performed with a 10 N-load cell for cooked samples and a 50 N-load cell for all other freezing and thawing combinations. Kramer shear tests ($n=6$) were carried out with a Kramer shear cell 10 blade (Fig. 1a) on 100 ± 1 g samples of green beans placed on the cell base. The deformation rate was 400 mm min^{-1} . Warner-Bratzler tests ($n=20$) were carried out with a Warner-Bratzler shear blade (Fig. 1b) on samples consisting of one bean, which was placed horizontally on the slotted blade insert. The bean was then cut across its longitudinal axis by the Warner-Bratzler blade at a deformation rate of 400 mm min^{-1} . The Kramer shear force-deformation curves were used to determine the maximum force, F_{KSC} (N per g of dry matter) and energy (area beneath the curve up to the maximum force), E_{KSC} (J per g of dry matter). The Warner-Bratzler shear force-deformation curves were used to determine the maximum force, F_{WB} (N), the maximum energy (area beneath the curve up to maximum force), E_{WB} (J) and the curve slope in the linear zone, S_{WB} (N mm^{-1}) (Fig. 1).

Dry matter content

Determinations were made on 20 g of green beans by drying samples in a microwave oven (Sanyo Electronic Co, Ltd. United Kingdom) with output power at 70% and set at position 2 (900W). Samples were weighed every 5 min until a constant weight was attained [22]. Ten determinations ($n=10$) were performed for each experimental unit.

Structural examination

Tissue structure was examined by SEM using a Hitachi model S-2500 microscope [23, 24]. Tissue samples were fixed in FAA {50 or 70% ethyl alcohol (90 ml), glacial acetic acid (5 ml) and formaldehyde (5 ml)} for 2 h and dehydrated in increasing concentrations of ethanol, from 70% to 100%. The samples were immersed once for 15 min in ethanol concentrations of 70, 80 and 90% and twice for 1 h in 100% ethanol. Finally, the specimens were preserved in acetone until processed in a critical-point drier, then mounted and sputter-coated with platinum (400-Å) in a P-S1 diode sputtering system metallizer. Photomicrographs were taken with a Mamiya camera using Ilford 6x9 cm FF-4 film. Films were processed following the standard method; magnifications were $\times 150$ and $\times 118$ (1 cm=66.7 μm , 1 cm=85.2 μm , respectively).

Objective colour measurements

Colour was measured in the surface of the green beans with a Minolta, CR 200b colorimeter (Minolta Corp, Tokyo, Japan). Thirty measurements per treatment ($n=30$) were recorded on the surface of the beans. The CIE $L^*a^*b^*$ colour notation system was applied to determine the parameters L^* , a^* and b^* ; where L^* indicates the luminescence (lightness), a^* means the colour axis from green to red and b^* the blue-yellow one. Cartesian coordinates were transformed into polar coordinates and the saturation index (r) and coloration (θ) were calculated [25-27]. Green bean chlorophylls and pheophytins ($n=2$) were determined by spectrophotometry (Unicam 8700 UV/VIS Spectrometer) [28].

Sensory analysis

An eight-member panel trained for frozen vegetables conducted sensory analyses for texture, taste and colour following a descriptive quantitative method adapted for green beans [29]. Scores were awarded on a scale of 1-5, in

which 1 indicated total absence of the sensory attribute and 5 a very definite attribute. Prior to testing, fresh and frozen green beans were cooked following the method described above [20].

Statistical analysis

Individual experiments were performed in duplicate, so that data represent the mean of two independent assays. Two-way analysis of variance was conducted to determine the combined effect of the different freezing and thawing rates on the mechanical properties and colour parameters of the beans, taking fresh green beans as control. The least significant difference test (LSD) was used for comparison of means, with a 99% confidence interval for quality parameters. One-way analysis of variance was conducted to determine the effect of the freezing rate on the mechanical properties, colour parameters and sensory attributes of the green beans after final cooking, taking cooked unfrozen green beans as control. The least significance difference test (LSD) was used for comparison of means, with a 99% confidence interval for objective parameters and 95% for sensory attributes. MSTAT Version 4.0 (Michigan State University, USA) was used in this study.

Results and discussion

Effect of freezing and thawing rates

Figure 2 shows the mean values and LSD (99%) of the several mechanical parameters derived from KSC and Warner-Bratzler tests for the green beans frozen and thawed at the rates stated in Materials and methods section. Freezing rates significantly affected the values of all the KSC (F_{KSC} , E_{KSC}) and Warner-Bratzler parameters (F_{WB} , S_{WB} , E_{WB}) ($P \leq 0.001$). Texture parameter values were highest in the samples frozen at the fastest freezing rate. This means that green beans frozen at $-70\text{ }^{\circ}\text{C}$ required greater force and energy for sequential compression, shear and

extrusion, and greater force and energy for shearing, than beans frozen at -35 and -24 °C. Also, beans frozen at -35 °C presented significantly higher mechanical resistance than those frozen at -24 °C (as evidenced from both KSC and WB maximum forces). Using programmed freezing, Fuchigami et al. [30-32] also found that -5 °C min⁻¹ was the optimum freezing rate to preserve texture and structure of carrots and Chinese cabbage. Of all the objective texture parameters, WB slope (S_{WB}) (Fig. 2b) presented the widest least significant difference (LSD) range when compared relatively (relative to the absolute values of the texture parameters) in relation to freezing rate effect. This result justifies that differences between WB slope values corresponding to the beans frozen at -35 and -24 °C were non-significant.

The thawing rate had a highly significant ($P \leq 0.001$) effect on the values of Warner-Bratzler maximum force and slope (F_{WB} , S_{WB}) (Fig. 2b) and a slight significant ($P \leq 0.05$) effect on KSC maximum force and Warner-Bratzler energy (F_{KSC} , E_{WB}) values. Parameter values were highest for air thawing at the slowest rate used ($R_t = 0.35$ °C min⁻¹). In relation to the thawing rate effect, F_{WB} presented the lowest LSD range when compared relatively, and therefore higher differences between thawed green beans could be found. All the parameters of the mechanical tests used in this research discriminate between freezing rates, but not all permit an assessment of the effect of thawing on these. KSC test did not explain the variations in textural characteristics resulting from the thawing process. Besides, other results suggested that any mechanical tests used should be independent of sample weight and volume, and the sample preparation should be as non-destructive as possible [33]. Although the Warner-Bratzler method is destructive in sample preparation, it is independent of the weight factor. On the other hand, the maximum force and energy parameters of the Warner-Bratzler test are dependent on sample volume. The slope parameter in this test, interpreted as increase in force/increase in deformation, has the advantage

of being less dependent on sample volume, and therefore **it should be recommended** that the best parameter for assessing the firmness of frozen green beans thawed at ambient temperature is the Warner-Bratzler slope (S_{WB}).

Overall analysis of the objective texture parameters obtained for the frozen/thawed samples and the fresh control (**Fig. 3**) showed highly significant differences ($P \leq 0.001$) in all cases. Means comparison test showed that experimental units frozen at the fastest rate and thawed either in air or by water immersion presented a KSC maximum force value (**Fig. 3a**) similar to that of fresh green beans. The slope derived from the Warner-Bratzler test (**Fig. 3b**) showed that only the green beans frozen at the fastest rate and air-thawed at the slowest rate did not differ significantly from fresh control. The highest mechanical resistance values were found in the beans frozen at the highest freezing rate and air thawed at the slowest thawing rate, confirming previous findings in potato [34]. When the two objective texture parameters (KSC maximum force and Warner-Bratzler slope) were compared, both presented similar relative least significant difference, so that the Warner-Bratzler test slope can be considered as the parameter that best reflects the firmness of fresh and frozen product under the given conditions, based on the advantage of being independent of sample weight and less dependent of sample volume as cited above. All the other textural parameters showed that the mechanical resistance of green beans frozen and thawed in any combination was significantly lower than that of fresh beans. Nevertheless, parameter values were higher in the samples frozen at the fastest rate and thawed either in air or in water. With the results from the samples frozen at -70 and -24 °C and thawed either in air or by water immersion, a softening coefficient was calculated for each objective texture parameter [24]. This is defined as the percentage decrease in the value of the parameter per °C min⁻¹ of reduction during the freezing process; it was calculated on the assumption that between the freezing rates reached in samples

frozen at -70 and -24 °C there was a difference of 4 °C per min. These coefficients are shown in Table 1. The highest coefficients were given by Warner-Bratzler slope (S_{WB}) and energy (E_{WB}) for water and air thawing respectively. In the case of the coefficient obtained for the Warner-Bratzler slope in the green beans thawed in water (4.725%), for example, this means that the texture of the beans frozen at -24 °C was almost 5 times softer than that of the beans frozen at -70 °C.

[Insert Table 1]

Brown [5] showed that different freezing rates (for freezing times of 1, 5, and 20 or more min) affected the tissue structure of green beans differently. Ice crystals grow larger at slower freezing rates, causing more damage to the cell structure. Fig. 4 shows microphotographs of outer parenchyma from either fresh or frozen green bean tissues (cv. *Win*). The fresh tissue (Fig. 4a) consists of relatively large cells with thick cell walls and large intercellular spaces, showing an undamaged cell structure with numerous chloroplasts and starch granules [35]. The existence of large intercellular spaces is consistent with previous findings, in contrast with the inner parenchyma [15]. The tissue frozen at -24 °C (1.5 °C min^{-1}) presents apparent cell wall breakdown, and the cell damage is quite appreciable (Fig. 4b,c). In both thawing modes, tissue tearing is very apparent with a high incidence of cell and cell wall rupture. Cells appear to be contracted and dehydrated by loss of internal cell pressure. To the contrary, there are practically no appreciable differences in morphology between fresh and frozen beans at -70 °C (5.4 °C min^{-1}), which exhibit an undamaged structure where the thick cell walls appear to be in a state of stress even after thawing (Fig. 4d,e). There are no appreciable differences between the morphology of beans thawed in air and by water immersion at any of the freezing rates (Fig. 4b,c and Fig. 4d,e). Microphotographs confirmed that the damage to the cell structure was more dependent on freezing rate than on the thawing mode, as several of the objective texture parameters indicated.

Both textural and structural results suggest that the freezing rate is inversely related to the degree of structural damage caused in a vegetable product, since the freezing rate determines the size and location of the ice crystals that form [6]. A slow freezing rate ($1.5\text{ }^{\circ}\text{C min}^{-1}$) alters the cellular structure of the beans, producing migration of water molecules from their normal location to the crystallization sites. The result is an increase in internal tension, giving rise to cell separation, collapse, and rupturing of cell walls and loss of turgidity after thawing. In contrast, quick freezing ($5.4\text{ }^{\circ}\text{C min}^{-1}$) increases the number and reduces the size of the ice crystals, reducing the mechanical damage to the tissue structure, as reflected in the texture of the product.

Fig. 5a illustrates the effect of freezing and thawing rates on the various colour parameters. The effect of both rates was highly significant for all colour parameters ($P \leq 0.01$) except saturation, where the effect of the freezing rate was only significant at ($P \leq 0.05$). Interaction between the two effects was not significant, which is indicative of the significance of each main effect studied independently of the other one. Luminescence values (L^*) were significantly lower at the lowest freezing rate and higher at the highest freezing rate, whereas coloration values (θ) were highest at the lowest freezing rate and lowest at the highest freezing rate, indicating that the quickest freezing rendered this bean variety less green than the slowest freezing. Freezing at an intermediate rate induced luminescence and coloration values intermediate between those found at extreme freezing rates. Luminescence (L^*) and saturation (r) values were highest when frozen beans were thawed in air, whereas coloration values (θ) were highest at the fastest thawing rate determined by water immersion, indicating that the slowest thawing rendered this bean variety less green than did the quickest thawing. Irrespective of the thawing medium, increased freezing rates produced a lighter-coloured product, confirming previous findings [36]. This author stated that

vegetable tissues that are frozen at slow rates and thawed commonly present darkening similar to that found after blanching. This fact is due to the changes caused in the slowly frozen tissue by the presence of extra-cellular water, which alters the reflectance surface as well as the light penetration depth. Overall analysis of the colour parameters for the frozen/thawed samples and the fresh control revealed highly significant ($P \leq 0.001$) differences in all cases. For the sake of brevity, plots are not shown. Comparison of the mean values of luminescence (LSD, 99%=2.54) and coloration (LSD, 99%=0.021) showed that frozen/thawed samples presented lower luminescence and higher coloration, i.e., had a darker green colour, than fresh control. Green beans frozen at $-24\text{ }^{\circ}\text{C}$ and thawed in water presented the lowest luminescence and the highest coloration values. With respect to saturation (LSD, 99%=0.169), all the frozen/thawed samples had significantly higher values than the fresh control, except the beans frozen at $-24\text{ }^{\circ}\text{C}$ and thawed in water; in this case, although they also presented higher saturation than the fresh control, the differences were not statistically significant. Such a result was to be expected when comparing a fresh product with its frozen/thawed counterpart. **However**, as noted above, with the quickest freezing and air thawing, the beans were less green than with the slowest freezing and the quickest thawing; the difference from the colour of fresh beans can therefore be expected to decrease. Results show that the quickest freezing and the slowest thawing had the least negative effect on the greenness of beans.

Nevertheless, freezing and thawing rates and their interaction had non-significant effects on chlorophylls *a* and *b* and pheophytins *a* and *b*, confirming previous findings (plots are not shown for the sake of brevity). Also, chlorophylls *a* and *b* were retained during thawing at ambient temperatures, with retentions averaging 93.78% and 93.41%, respectively [17]. In the

frozen/thawed beans, chlorophyll *a* ranged between 49.801 and 50.918 mg/100 g dry matter, while chlorophyll *b* ranged between 19.241 and 20.576 mg/100 g dry matter. Pheophytin *a* and *b* contents ranged between 4.534 and 5.273 mg/100 g dry matter and 4.678 and 5.794 mg/100 g dry matter respectively. Compared with fresh control, the frozen/thawed green beans presented significantly greater ($P \leq 0.01$) degradation of chlorophyll *a* (Fig. 5b); no significant differences were found between fresh and processed green beans as regards any of the other pigments. The pigment contents in different green vegetables evolve very differently during freezing. The effects of freezing and vacuum packing on the pigment contents in green beans (blanched and unblanched) over the course of 12 months were evaluated [10]. In frozen beans, chlorophylls *a* decreased considerably during month 1 but were stable over the next 11 months.

Effect of freezing rate in cooked green beans

Figure 6 shows mean values and LSD (99%) of the various objective mechanical texture parameters derived from KSC and Warner-Bratzler tests for green beans frozen at the three rates and cooked, in comparison with green beans cooked directly without previous freezing. The freezing rate had a highly significant ($P \leq 0.001$) effect on the KSC parameter values (Fig. 6a) and on the maximum force and slope derived from the Warner-Bratzler test (Fig. 6b). Again, green beans frozen at $-70\text{ }^{\circ}\text{C}$ required greater force and energy for sequential compression, shear and extrusion, and greater force for shear than did beans frozen at -35 and $-24\text{ }^{\circ}\text{C}$, or even cooked without freezing. Only in the case of the Warner-Bratzler slope (S_{WB}), was there a non-significant difference between the samples cooked after freezing at the fastest rate and the directly-cooked

control. The results indicate that the fastest freezing produced the least damage to green bean; this effect was detectable even after cooking the product. Of the objective parameters used to detect the effect of the freezing rate on thawed and cooked samples, KSC maximum force (Fig. 6a) and Warner-Bratzler slope (Fig. 6b) presented the lowest values of relative LSD, which means that more significant differences can be found using these parameters, and the latter (S_{WB}) having the advantage of being independent of sample weight.

Fig. 7 shows microphotographs of outer parenchyma of either directly-cooked beans or beans cooked after freezing at the three rates. Note how the direct relationship between the freezing rate and the degree of structural damage is quite evident in the beans frozen and subsequently thawed by traditional boiling in water. Unlike the cooked control (Fig. 7a), freezing caused rupturing of some cell walls, which displayed fissures resulting from internal tensions in the tissue caused by the change of phase from water to ice. However, increasing the freezing rate (Fig. 7b-d) reduced the damage induced by freezing. Gelatinised starch granules are visible in all the microphotographs of samples after cooking. It is known that both freezing and cooking processes soften green bean tissue although in different manners. While freezing causes cell wall rupture, cooking mainly causes solubilisation of intercellular pectin and extensive cell separation. Some cell rupture was apparent in the cooked control (Fig. 7a). The rupture of cells as a consequence of boiling in water appeared to be due to distension of the cell wall [37].

Overall analysis of the objective colour parameters from the frozen and cooked samples and the cooked control (Fig. 8) revealed highly significant differences in luminescence (L^*) and coloration (θ) ($P \leq 0.001$), and significant differences in saturation (r) ($P \leq 0.01$). Freezing prior to cooking significantly increased the luminescence of the beans with respect to the unfrozen control,

while the differences between samples frozen at different rates were not significant. Frozen and cooked beans presented the same colour saturation, while the samples frozen at $-35\text{ }^{\circ}\text{C}$ presented the highest saturation value. The coloration of all the frozen and cooked beans was significantly weaker than that of the directly-cooked beans, indicating that freezing prior to cooking produces lighter-coloured beans than does direct cooking. When beans were thawed after freezing, a faster freezing rate produced lighter-coloured beans, while when beans were cooked after freezing; a faster freezing rate produced darker-coloured beans. Samples frozen at $-24\text{ }^{\circ}\text{C}$ and cooked presented the lowest greenness, which may be ascribed to chloroplast degradation due to superior cellular damage caused by slow freezing; this can cause pigments to migrate to the inner parenchyma, hence producing a lower chlorophyll concentration in the outer epidermis, hypodermis and outer parenchyma.

Comparison of the pigment contents of the frozen and cooked samples with those of cooked control (data not shown) showed non-significant differences for the different pigments ($P > 0.05$). Chlorophyll *a* ranged between 15.180 and 15.999 mg/100 g dry matter, whereas chlorophyll *b* ranged between 13.344 and 14.399 mg/100 g dry matter. With respect to pheophytins *a* and *b*, values ranged between 39.639 and 41.181 and between 12.144 and 13.072 mg/100 g dry matter respectively. Note that in the case of pigment values of frozen samples thawed at ambient temperature, cooking decreased chlorophyll content, mainly chlorophyll *a*, and noticeably increased both types of pheophytin, mainly pheophytins *a*. Pheophytisation is enhanced by the increase in H^{+} concentration, in which the centre magnesium of chlorophylls is replaced by hydrogen, forming pheophytine. Pheophytine gives an olive-brown colour to green beans [17].

The effect of freezing rate on the average scores given by the panellists to all the sensory attributes in the cooked beans was not significant ($P > 0.05$). In other words, in sensory terms panellists found the colour, texture and flavour of the directly-cooked beans and of beans previously frozen at any freezing rate, to be equally acceptable. With respect to colour attributes, the cooked green beans scored intermediate for greenness (average score 3), with high uniformity (average score 4), no off-colours (average score 5) and with a moderately low shine (average score 3). Also, the cooked beans scored as acceptable for tenderness and firmness (average scores 4), with no fibrosity (average score 1), moderate for sweet taste (average score 3), high for authentic taste (average score 4) and low for a bitter or off-taste (average score 1). The results for the effect of the freezing rate on sensory texture attributes (tenderness and firmness) and sensory colour attributes (greenness, shine, uniformity) of cooked beans do not corroborate the results for the objective texture (F_{KSC} , E_{KSC} , F_{WB} , S_{WB}) and colour (L^* , r , θ) parameters, which indicated a significant difference as shown above. These discrepancies demonstrate the effect adverse of cooking on sensory attributes, masking the different structural alterations caused by rapid or slow freezing. Sensory properties are generally used to quantify the shelf-life of frozen vegetables, and properties such as the perception of colour, texture and flavour have been used to quantify quality as perceived by the consumer. However, Martins and Silva [1] found that sensory parameters may not be the most suitable shelf-life limiting factors in frozen vegetables, as our results confirm. To the contrary, they corroborate other studies in which objective texture analysis methods have been successfully used to detect the effect of rapid freezing rates, even after cooking in the case of green beans [7].

Conclusions

Tissues of green beans frozen at the fastest rate ($5.4\text{ }^{\circ}\text{C min}^{-1}$) and air-thawed at the slowest rate ($0.35\text{ }^{\circ}\text{C min}^{-1}$) presented the highest mechanical resistance, with the freezing rate causing more mechanical damage to the tissue structure than the thawing rate. Objective texture tests detected the positive effect on the texture of the green beans of increasing the freezing rate, even after cooking. Cell damage caused to frozen and cooked beans correlated inversely with the freezing rate, i.e., a higher freezing rate reduced the damage to texture and tissue structure. All the parameters of the mechanical tests used in this research discriminate between beans frozen at different freezing rates, but not all permit an assessment of the effect of thawing on the tissue. The slope is the parameter of the Warner-Bratzler test curve that best reflects the changes occurring in the texture and structure of the tissue as a result of freezing in the case of both thawed and cooked beans, being independent of the sample weight. Frozen and thawed green beans were darker than fresh control, whereas frozen and cooked beans were lighter than directly cooked controls. When the beans were thawed, a higher freezing rate reduced the greenness of the samples; water thawing produced darker samples than air-thawing, while in cooked beans a higher freezing rate increased greenness.

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Figure legends

Fig. 1 Mechanical force-deformation curves and test cells used in the rheological characterisation of green beans. **a** 10 Kramer shear cell. **b** Warner-Bratzler shear blade

Fig. 2 Effect of freezing and thawing rates on the mechanical properties of green beans (cv. *Win*). **a** KSC test, **b** Warner-Bratzler test

Fig. 3 Comparison between fresh and frozen/thawed green bean mechanical properties **a** KSC test, **b** Warner-Bratzler test

Fig. 4 Microphotographs of fresh and frozen green bean tissues (cv. *Win*) thawed at ambient temperatures **A** Fresh control, **B** Frozen at -24 °C and air thawed; **C** Frozen at -24 °C and water thawed, **D** Frozen at -70 °C and air thawed; **E** Frozen at -70 °C and water thawed (1cm=66.7 µm) *CW* cell wall, *IS* intercellular space, *St* starch

Fig. 5 a Effect of freezing and thawing rates on the Cartesian and polar colour coordinates, **b** Comparison between fresh and frozen/thawed green bean chlorophylls and pheophytins

Fig. 6 Effect of freezing rate on the mechanical properties of cooked green beans (cv. *Win*). **a** KSC test, **b** Warner Bratzler test

Fig. 7 Microphotographs of cooked and frozen green bean tissues (cv. *Win*) thawed by direct cooking **A** Cooked control (1cm=66.7 µm); **B** Frozen at -24 °C and cooked (1cm=85.2 µm), **C** Frozen at -35 °C and cooked (1cm=85.2 µm), **D** Frozen at -70 °C and cooked (1cm=85.2 µm)

Fig. 8 Effect of freezing rate on the Cartesian and polar colour coordinates of cooked green beans (cv. *Win*)

Table 1 Softening coefficients of objective texture parameters per °C min⁻¹ reduction during freezing process

<i>Thawing mode</i>	F_{KSC}	E_{KSC}	F_{WB}	S_{WB}	E_{WB}
	(N/g d.m)	(J/g d.m)	(N)	(N mm ⁻¹)	(J 10 ⁻³)
Air at +20 °C	2.329	1.883	3.296	3.877	4.661
Water at +20 °C	3.030	2.392	3.062	4.725	2.176

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8