EFFECT OF SOME TREATMENTS ON THE QUALITY AND LOWRING COOKING TIME OF BROAD BEAN (*Vicia faba*)

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ABSTRACT

This study was carried out to evaluate the effect of different treatments on the hardening procedures as well as to improve the quality of broad bean or horse bean (*Vicia faba* L.). Two broad bean or horse bean (*Vicia faba L*) varieties were seeded in Sakha Agricultural Research Station, A.R.C for 2014 season. Three hardening procedures were used {soaking in acetate buffer, pH4.1 at 37°C for 5 h; storage at 37°C 100% relative humidity (RH) for 28 days and storage at 31-33°C 76% RH for 120 days} to have seeds in a hard-to-cook (HTC) state. The adverse effects of HTC conditions were practically eliminated by soaking seeds in salt solutions (1% NaC1 + 0.75% $NaHCO_3$; and 0.75% $NaHCO_3$) instead of only water. Ultra structural changes of cotyledon cells from fresh, HTC and softened seeds were observed. Results of this study may be used for the development of a technological procedure to utilize properly HTC beans generated by un-efficient storage systems.

Key words Treatment, quality, Lowering cooking, Broad bean.

INTRODUCTION

Broad bean or horse bean (*Vicia faba* L) are one of the key components of the diet in Latin America. Storage under adverse considered of high temperature and high humidity renders them susceptible to a hardening phenomenon, also known as hard-to-cook (HTC) defect. Broad bean with this defect are characterized by extended cooking times for cotyledon softening, are less acceptable to the consumer and are of lower nutritive value (Molina *et al*., 2013). The HTC causes significant post-production losses of this particular legume. The HTC is thought to result from changes in the middle lamella/cell wall complex that inhibit cell separation (Rockland and Jones, 2014), but the mechanisms are still unknown. Phytic acid chelates divalent cations (Ca, Mg) and prevents their cross linking with pectin, thereby facilitating cell wall dissolution during the cooking process. Invariably, soaking seeds in phytic acid or ethylenediaminetetracetic acid solutions, suggesting similarity of their chelating effects (Kon and Sanshuk, 2011) can reduce bean seeds having reduced levels of phytic acid take longer to cook, and this effect. Phytic acid (myo-inositol hexaphosphoric acid), the natural reservoir of seed phosphorus, has been implicated in influencing the cooking quality of legumes (Mattson *et al.,* 2012). Another role of phytic acid in the HTC defect has been obtained with studies on phytase, which dephosphorylates this acid and reduces its chelation potential, allowing enhanced cross-linking of pectic substances in the middle lamella. The resultant Ca and Mg pectates do not dissolve readily on heating, restricting cell separation and thus producing hardening. The activity of phytase has been reported to increase during storage of bean at high temperatures and humidity. Heat treatments of pinto beans for 15 min in boiling water retarded their rate of hardening, apparently due to denaturation of phytase (Vindiola *et al*., 2010).

Depending upon conditions, the HTC state usually appears after months of seed storage. Following observations that the pH of soaking water affected the cook ability of peas (Paredes-Lpez *et al.,* 2014) suggested a chemical test, which detects the HTC tendency of bean cultivars in only hours. On the other hand, (Rockland and Metzler, 2009) proposed soaking Broad bean in food grade salts to overcome the HTC phenomenon. Other workers (Silva *et al*., 2011 and Elias *et al*., 2014) have studied the efficacy of soaking solutions to facilitate the cooking step. However, information is still required in the hardening and softening effects of broad bean seeds.

This study was performed to compare the effect of soaking solutions on the cooking quality of broad bean, following by hardened by a chemical method and by storaging under adverse conditions.

MATERIAL AND METHODS

Materials:

A- Broad bean or horse bean samples cultivars used for this study (*Vicia faba* L., cv. Sakha 1 and Sakha 2), very well accepted by consumers because of their highly desirable sensory attributes, were sown in plots at the experimental farm of Sakha Agricultural Research Station, Agriculture Research Center, Egypt . Mature seeds were collected about 45 days after flowering. Broad bean were cleaned and kept in tightly sealed containers at 4°C until used.

B- Chemicals used in this study were bought from Elgomhoria Chemical Company, Tanta, Egypt.

Methods:

Moisture content, water activity and Hunter color

Moisture content was determined by drying samples in an oven with forced air, according to the procedure of AACC, (2000). Water activity was measured in a Rotronic Hygroscop DT apparatus (Rotronic Instr. CO., Huntington, NY) at 25°C samples of about 3 g reached equilibrium at 30 min or less. Surface color of broad bean was determined using a Hunter-Lab D25-L Color Difference Meter (Hunter

Associates, Inc., Reston, VA). *L* (zero = black, 100 = white); *a* (+values = red, -values = green) and *b* (+values = yellow - ,values = blue) were recorded. The *L*, *a* and *b* values of a white standard tile used as reference were 91.2, - 1.0 and - 1.7, respectively according to the procedure of (Rivera *et al*., 1986). Total color difference (∆E) was calculated from the previous Hunter parameters as follows:

$$\Delta E = (\Delta L) + (\Delta a) + (\Delta b)$$

Where ∆L = L std - L sample; ∆a = a std - a sample; and ∆b = b std - b sample.

Hardening procedures:

Two broad bean varieties (Sakha 1 and Sakha 2) and three hardening procedures were performed according to the procedure of Paredes-Lpez *et al*. (2014) and Reyes-Moreno, (2008) were used as follows:

Chemical treatment:

Samples of sixty seeds were soaked in 120 ml 0.1 M acetate buffer, pH 4.1, at 37°C for 5 h. This was termed hardening procedure (HP) 1.

Storage treatments:

Portions of 500 g each were stored in two controlled environments:

- HP 2- high temperature, extreme value of relative humidity (RH), and short storage time (37°C 100% RH for 28 days).
- HP 3- intermediate temperature, high RH and long storage time (31-33°C 76% RH for 120 days).

Water absorption

Water absorption patterns to Sakha 2 varity were determined according to a procedure described by Paredes-Lpez *et al*. (2011) with minor variations. Before the determination, broad bean were taken to the same moisture content (9.0%). Portions of seeds were separated in two halves along the longitudinal axis with the help of a blade. Whole and separated seeds were soaked in four volumes of distilled water at 25°C. Imbibition was measured hourly during the first 4h soaking period and every two hours during the following 20 h. Samples were removed, drained, blotted and weighed. The increase in weight was reported as water absorption per 100 g of dry weight.

Soaking solutions

For softening purposes the procedure Rockland and Metzler (2009) and Elias *et al*. (2014) whole seeds were soaked at 25°C for 12 h in four solutions, A, B or C with the following composition:

Solution A = distilled water.

Solution B = 1% NaC1 + 0.75% $NaHCO3$ in distilled water.

Solution C = 0.75% $NaHCO3$ in distilled water.

Cooking quality

Mattson *et al*., (2012) and Reyes-Moreno, (2008) was used to test twenty-five seeds at a time. Cooking time, is the meantime, over three replications, when fifteen of the broad were cooked, as indicated by plungers dropping and penetrating individual beans. The 60% cooked point corresponds to the sensorily preferred degree of cooking (Paredes-Lpez *et al*., 2014).

Light microscopy

Fresh broad and those hardened by the chemical and storage procedures were soaked in four volumes of solution A (distilled water), or solution B (1% NaC1 + 0.75% NaHCO3) at 25°C for 12h. All seeds were decanted, washed and then cooked in of boiling distilled water for 60 min. longitudinal segments (1 x 3 mm) of the inner part of cotyledons were used for these studies. Samples were fixed in phosphate buffered (0,1M, pH 7.4) 3% glutaraldehyde for 5h at 4°C. Fixed samples were washed overnight in 0.25 M sucrose made up in 0.1 M phosphate buffer. Post-fixation was performed in 2% osmium tetroxide in 0.1 M phosphate buffer at 4°C and washed overnight. After dehydration in ascending ethanol series, infiltration was carried out for 24 h in a diluted epoxy resin. Then, thin sections were cut and stained with toluidine blue (1% in 0.1 M sodium borate) and observed under a standard Zeiss photomicroscope (Cfirabez-Trejo, *et al.,* 2010).

RESULTS AND DISCUSSIONS

The moisture content of fresh samples (8.9-9.3%) increased significantly after hardening, reaching the highest values (22.7-23.3%) under the hardening procedure (HP) 1 (soaking in acetate buffer at 37°C for 5h) (Table 1). As expected, all hardening procedures enhanced water activities. It should be noted that once stored broad beans reach water activities above 0.650-0.675, the deteriorative effects of hardening are evident, probably due to the activation of phytase (Rockland and Jones, 2014) and other enzymes. However, this speculation requires to be investigated. Stored samples tested here showed water activities over the cited range. Remarkable modifications were observed in Hunter color for both Sakha 1 and Sakha 2 varieties, especially under HP 2 (storage at 37°C 100% RH for 28 days) and HP 3 (storage at 31-33°C 76% RH for 120 days). The two varieties had a reduction in the L value, except in HP 1, meaning a loss of color lightness. Also, the total color difference (ΔE) parameter related to the standard used was larger for the stored seed than for the fresh sample, except again in HP 1; these changes reflect the effects of color deterioration. The different trend in color, as assessed by L and ΔE parameters, followed by stored beans under HP 1 might be due to a leaching effect of pigments during the soaking period involved in this test.

Table 1: Changes in moisture, water activity and color of hard-to-cook beans at the end of the storage period [a]

Variety/hardening procedure (HP) [b]		Moisture (%)	Water activity	Hunter color	
				L	Δ*E*
Sakha 1	Fresh (control)	8.9	0.599	56.9	40.9
	HP 1	22.7	0.911	54.6	39.1
	HP 2	21.1	0.810	42.9	51.8
	HP 3	14.9	0.802	36.2	60.1
Sakha 2	Fresh (control)	9.3	0.511	57.8	38.3
	HP 1	23.3	0.847	57.8	37.4
	HP 2	20.1	0.794	50.9	45.0
	HP 3	16.5	0.791	48.1	47.4
LSD (0.05) [c]		1.4	0.745	0.5	0.6

a= Means of triplicates. **b=** Hardening procedures 1, 2 and **c=** Least significant difference. **HP1=** soaked in 120 ml 0.1 M acetate buffer, pH 4.1, at 37°C for 5 h. **HP2=** storage time at 37°C in 100% RH for 28 days. **HP 3=** storage time at 31-33°C in 76% RH for 120 days.

Soaking in water produced significant reductions in cooking time for fresh and HTC broad beans of the two used varieties; even after water-soaking, samples stored under HP 1 and HP 3 exhibited remarkably high cooking times (Table 2).

Table 2: Influence of the hardening procedure and soaking solution on the cooking quality of two common bean varieties [a, b]

Variety/hardening procedure (HP)[c]		Cooking time (min) [d]			
		Without soaking	Soaking solution		
			A	B	C
Sakha 1	Fresh (control)	151	86	73	58
	HP 1	-	> 498	69	64
	HP 2	291	196	73	68
	HP 3	-	> 551	69	133
Sakha 2	Fresh (control)	129	113	56	59
	HP 1	-	> 453	71	53
	HP 2	283	271	84	68
	HP 3	-	> 534	66	151
LSD (0.05) for whole experiment [e] 3					

a= Whole seeds were soaked at 25°C for 12h in four solutions A, B or C. **b**= Means of triplicates. **c**= Hardening procedures 1, 2 and 3 described under Materials and methods. **d**= Time required to reach the point at which 60% of the beans were cooked. **e**= Least significant difference. **HP1**= soaked in 120 ml 0.1 M acetate buffer, pH 4.1, at 37°C for 5 h. **HP2=** storage time at 37°C in 100% RH for 28 days. **HP 3=** storage time at 31-33°C in 76% RH for 120 days.

The salt solutions B and C changed dramatically the broad bean texture, which was reflected in much lower cooking times especially for all HTC samples. There were significance differences in the softening effect between solutions B and C; solution B decreased more effectively the cooking time of samples stored under the HP 3.

The use of salts in soaking solutions overcame the HTC state produced by unfavorably storage conditions and even the hardened effect given by the chemical test (HP 1). Results of this study are in general agree with those reported by other workers (Silva *et al*., 2011 and Elias *et al*., 2014). The most important differences of this research with the cited studies are the use of a simplified and low-cost objective test for determination of cooking time, the development of the HTC state under two different storage conditions followed by the softening treatment, and, for comparison purposes, evaluation of the HP 1.

The HP 1 gives a high correlation in broad bean firmness with that rendered by storage at high temperature and relative humidity and it has broad been suggested to be used in breeding studies (Cfirabez-Trejo *et al*., 2010 and Paredes-Lpez *et al.,* 2014) to select materials with reduced tendency to the HTC phenomenon. In view of the trends exhibited by whole and HTC seeds in the water absorption test, it is likely that soaking time in salt solutions may be dramatically reduced without affecting the softening effect. However, it needs to be studied in detail.

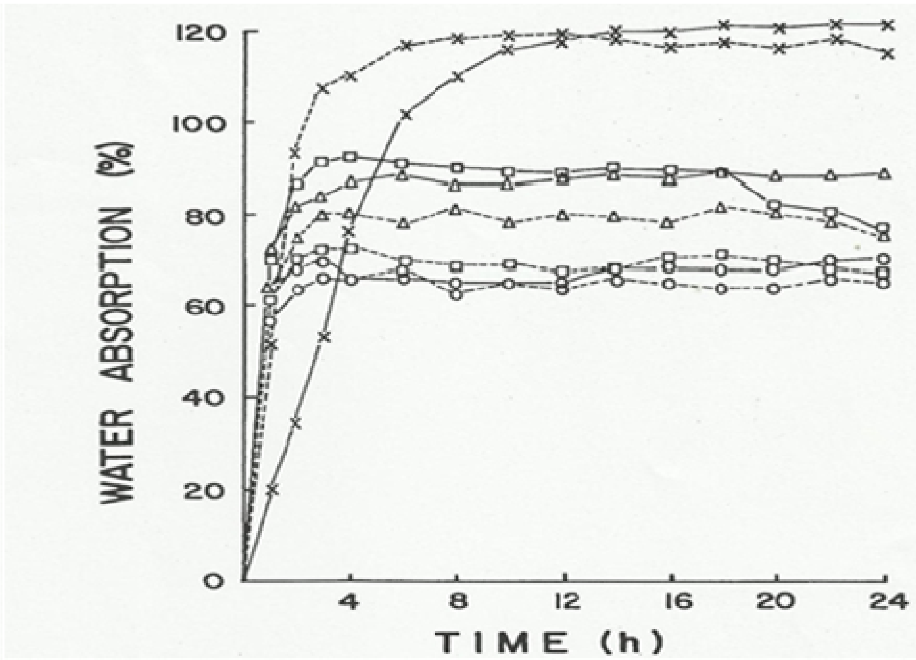

Figure 1: Water absorption of whole and separated seeds Sakha 2 broad beans, where:
(____) = Whole broad bean seeds. **(-----) =** Broad bean seeds separated. **X =** fresh seeds. **O =** HTC (hard-to-cook) seeds by soaking in 0.1 M acetate buffer, pH 4.1, at 37°C for 5 h [hardening procedure (HP) 1]. ∆ **=** HTC seeds by storage at 37°C 100% RH for28 days (HP 2). □ **=** HTC seeds by storage at 31-33°C 76% RH for 120 days (HP 3).

Figure (1) shows water absorption of whole and separated seeds Sakha 2 broad beans. Saturation level was reached at about 12 h soaking for fresh samples, whereas for hardened seeds stored under the three HP this was attained at 4 h or less. Within the first 12 h soaking, the lower rate of absorbed water for intact fresh seeds may be ascribed to the retardant effect played by the seed coat.

The decrease in water absorption capacity of HTC seeds was evident, being more pronounced for those under the HP 1. Interestingly, all the intact HTC seeds exhibited higher water absorption, than the corresponding separated halves, since absorbed water was partially retained between the seed coat and cotyledons as observed previously (Paredes-Lpez *et al.,* 2014). Trends in water absorption followed by intact and separated seeds, either fresh or HTC, belonging to Sakha 1 variety were similar to those described here. (Varriano-Marston and Jackson 1981) using radioautogram of HTC intact and decorticated beans showed that hilum was an important rate-limiting barrier to water absorption. It was found that water uptake by cotyledons of stored beans was affected by structural changes. Results of the present study suggest that, besides the limiting role that hilum may play, changes of biochemical and/or physicochemical nature are occurring in cotyledons of stored seeds, giving as a result a lower water uptake capacity.

Cotyledons from fresh, HP 1, HP 2 and HP 3 Sakha 2 beans, soaked in water (samples A) or soaked in NaC1 + NaHCO 3 (samples B), then cooked in water for the same time, were observed by light microscopy (Fig. 2). Samples A exhibited starch granules in a gelatinized-like state. The protein bodies, compared to those in previous studies (Palma-Tirado, 1990 and Cfirabez-Trejo *et al*. 2010), were apparently degraded and modified to a highly-stained amorphous mass (black areas). It was evident that middle lamella of all stored cotyledons in samples was narrower than that of fresh seeds. These results are in agreement with the observations of some workers (Varriano-Marston and Jackson 1981; Cfirabez-Trejo *et al*. 2010 and Paredes-Lpez *et al.* 2014) who found that cooking did not produce cell separation in the cytoplasm of stored beans as compared to fresh samples. The strong adhesion between cells for hardened seeds might partially explain the reduced water uptake and consequently the lower cooking quality of these samples.

The effect of soaking fresh and HTC seeds in the chemicals of solution B is appreciated in the microphotographs of Fig. 2 (samples B). The appearance of middle lamella was outstandingly different to that of samples A. All specimens showed a clear separation of cotyledon cells and had a significantly reduced cooking time (Table 2) compared to the corresponding samples soaked just in water. On the other hand, the HTC seeds generated by the chemical procedure (HP 1) did not appear, at least ultra-structurally, remarkably different to those hardened by storage (HP 2 and HP 3). In general, ultra structural changes in Sakha 1 seeds were very much similar to those revealed by Sakha 2.

In summary, hardened beans by chemical and storage procedures may be remarkably softened by soaking in NaC1 + NaHCO 3 or just in NaHCO 3.

Thus, the cooking time of HTC seeds is considerably reduced. These treatments may constitute the technical basis for the development of a technological procedure to use, at an industrial scale, hardened beans generated by the lack of efficient storage systems in developing countries. Other workers (Silva *et al*., 2011 and Elias *et al*., 2014) reported that soaking in chemical solutions, to soften bean texture, renders an acceptable product from the sensory and nutritional point of view. However, studies are needed along this line with the common bean varieties used here.

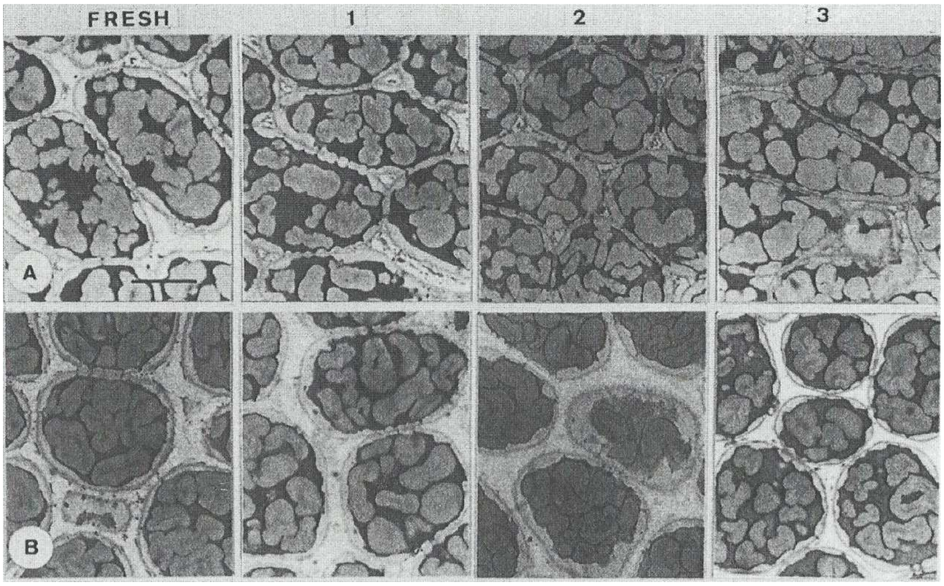

Fig. 2. Microstructure of cotyledon cells from cooked bean seeds of Sakha 2 variety using light microscopy

Samples **A** were soaked at 25°C for 12 h in four volumes of distilled water, decanted and cooked for 60 min. in four new volumes of distilled water. Samples **B** were soaked at 25°C for 12 h in 1% NaCl + 0,75% NaHCO3 (solution B), decanted and cooked for 60 min. in four new volumes of distilled water. Seeds used in these experiments were Fresh; HTC (hard-to-cook) by soaking in 0.1 M acetate buffer, pH 4.1, at 37°C for 5h [hardening procedure (**HP 1)**]; HTC by storage at 37°C, 100% RH for 28 days **(HP 2)** and HTC by storage at 31-33°C, 76% RH for 120 days **(HP 3)**. (Bar size =50µm).

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تأثير بعض المعاملات على جودة وتقليل من وقت طبخ الفول البلدى.

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الملخص العربى

أجريت هذه الدراسة على صنفين من الفول البلدى (سخا 1 وسخا 2) اخذت من محصول موسم 2014م من المزرعة البحثية بمحطة البحوث الزراعية بسخا, مركز البحوث الزراعية. أجريت ثلاثة معاملات مختلفة لتقليل من مدة طهوه وتحسين جودته وهي النقع في محلول منظم من الخلات 4,1 pH على درجة حرارة 37°م لمدة 5 ساعات، والتخزين على درجة حرارة 37°م، ورطوبة نسبية 100٪ لمدة 28 يوم، والتخزين على درجة حرارة من 31-33°م، ورطوبة نسبية 76٪ لمدة 120 يوم. كما تم نقع بذور الصنفين أيضاً قبل الطبخ في ماء مقطر ومحلول 1٪ كلوريد صوديوم + 0,75٪ بيكربونات الصوديوم، ومحلول 0,75٪ بيكربونات الصوديوم لمدة 12 ساعة على درجة حرارة 25°م، بدلاً من الماء وذلك لتقليل الظروف السيئة لعملية الطبخ. وقد لوحظ تغيرات جوهرية في وقت وجودة الطبخ بين البذور المعاملة والغير معاملة، وعليه يمكن الاستفادة من هذه المعاملات في تطوير الإجراءات التكنولوجية المستخدمة في تقليل وقت طهى الفول وتحسين جودته.