Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Abd El-Gawad IA et al., J Nutr Food Sci 2015, 5:5
DOI: 10.4172/2155-96001000411

Commentary Open Access

Preparation Technique of Soymilk-Based Yoghurt and it's Relation to Soybean Varieties and Anti-Nutritional Factors

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Rec Date: July 25, 2015; **Acc Date:** Aug 28, 2015; **Pub Date:** Sep 09 2015

Abstract

The effect of soybeans varieties and the extraction ratios on the chemical composition of the resulting soymilk, additionally the effect of the preparation technique of soymilk and soy-yoghurt on the trypsin inhibitors, urease activity and phytic acid contents were investigated. Soybean seed varieties "Clark and Crawford" and their structural components were analyzed for major chemical composition and soluble carbohydrates. Moreover, the changes in the soybean anti-nutritional factors affected by preparation technique of soymilk and soy-yoghurt were determined. Cotyledon of Clark variety contained significantly ($p<0.05$) lower moisture and total soluble sugars in particularly stachyose compared with that their values of Crawford variety. Soymilk constituents increased with decreasing the amount of water used in the extraction process (1:10 to 1:4 cotyledon: water). Soymilk processing under alkaline conditions showed completely destroyed trypsin inhibitors and urease activity and markedly reduced the amount of phytic acid in soymilk and consequently soy-yoghurt. The results showed that the effectiveness of soybean varieties and the processing technique used in the preparing of soy milk and soy-yoghurt in removing the negative effect of the anti- nutritional factors.

Keywords: Anti-nutritional factors; Soybean varieties; Soymilk; Soy-yoghurt; Processing technique

Introduction

Due to the current worldwide shortage of food, attempts have been made to find alternative sources of protein, particularly for the developing countries, where malnutrition exists. As a result, for that, shifts from animal to vegetable sources of protein have increased significantly. However, soybean is plentiful, relatively inexpensive and excellent source of energy, oil and protein. In addition, it supplies fairly good quantity of vitamins and minerals such as calcium, phosphorus and iron. Soybeans also have beneficial effects on human health, being very low in sodium, cholesterol, saturated fatty acids, but rich in polyunsaturated fatty acids and dietary fiber with both soluble and insoluble fiber soybean [1,2].

Towards such advantages, some efforts have been devoted to exploiting it for the manufacture of more acceptable and palatable food products. One of the simplest methods for converting soybeans to a high protein food is to extract the beans with water to produce a beverage known as soy-milk. Traditionally soymilk is made by soaking the soybeans, grinding them in water, cooking the slurry and then filtering to remove sludge [3]. Unfortunately, soymilk produced in this manner has a distinct beany flavor and contains large amount of soluble carbohydrates (stachyose & raffinose) causing flatulence and anti-nutritional factors that may cause difficulties for humans. The soybean anti-nutritional factors include trypsin inhibitors (TI), urease enzyme and phytic acid. Soybean trypsin inhibitors depresses growth and causes enlargement of the pancreas in animals and it also reduces the digestibility of protein, increases the sulfur amino acid requirement [4-8].

Urease enzyme degrades urea to form ammonia a very toxic compound, whereas phytic acid can decrease the availability of divalent cations, such as calcium, zinc and iron [9,10]. Because of very limited acceptance of soymilk produced by the traditional process, many attempts have been made to develop a bland soymilk, which contains no or less amount of soluble carbohydrates and nutritional factors [9,11-13].

On the other hand, the chemical composition of soymilk varies and depends upon varieties of soybeans [8] and processing conditions particularly the ratio of soybean to water [14]. Therefore, the possibility of selecting a proper soybean variety with high solids and decreased soluble carbohydrates should be explored and encouraged in the manufacture of soymilk and its products.

The aim of this study was to investigate the effect of soybeans varieties and the extraction ratios on the chemical composition of the resulting soymilk as well as to determine the effect of the preparation technique of soymilk and soy-yoghurt on the trypsin inhibitors, urease activity and phytic acid contents.

Materials and Methods

Soybean varieties

Two varieties of soybeans: Clark and Crawford were obtained from Crops Research Department, Field Crops, Agriculture Research Centre, Ministry of Agriculture, and Giza, Egypt. Representing samples of dry mature soybean seeds were cleaned to remove any foreign materials and immature beans and ground as whole or after dehulling (cotyledon and hull). Ground whole soybean seeds, cotyledons and hulls were kept in polyethylene bags.

Soymilk preparation: Bland and Smooth soymilk was prepared from each soybean variety according to the procedure of Tanteeratarm, Nelson & Wei [15] at the pilot plant of Food Technology Research Institute, Ministry of Agriculture, and Egypt. The method described in Figure 1.

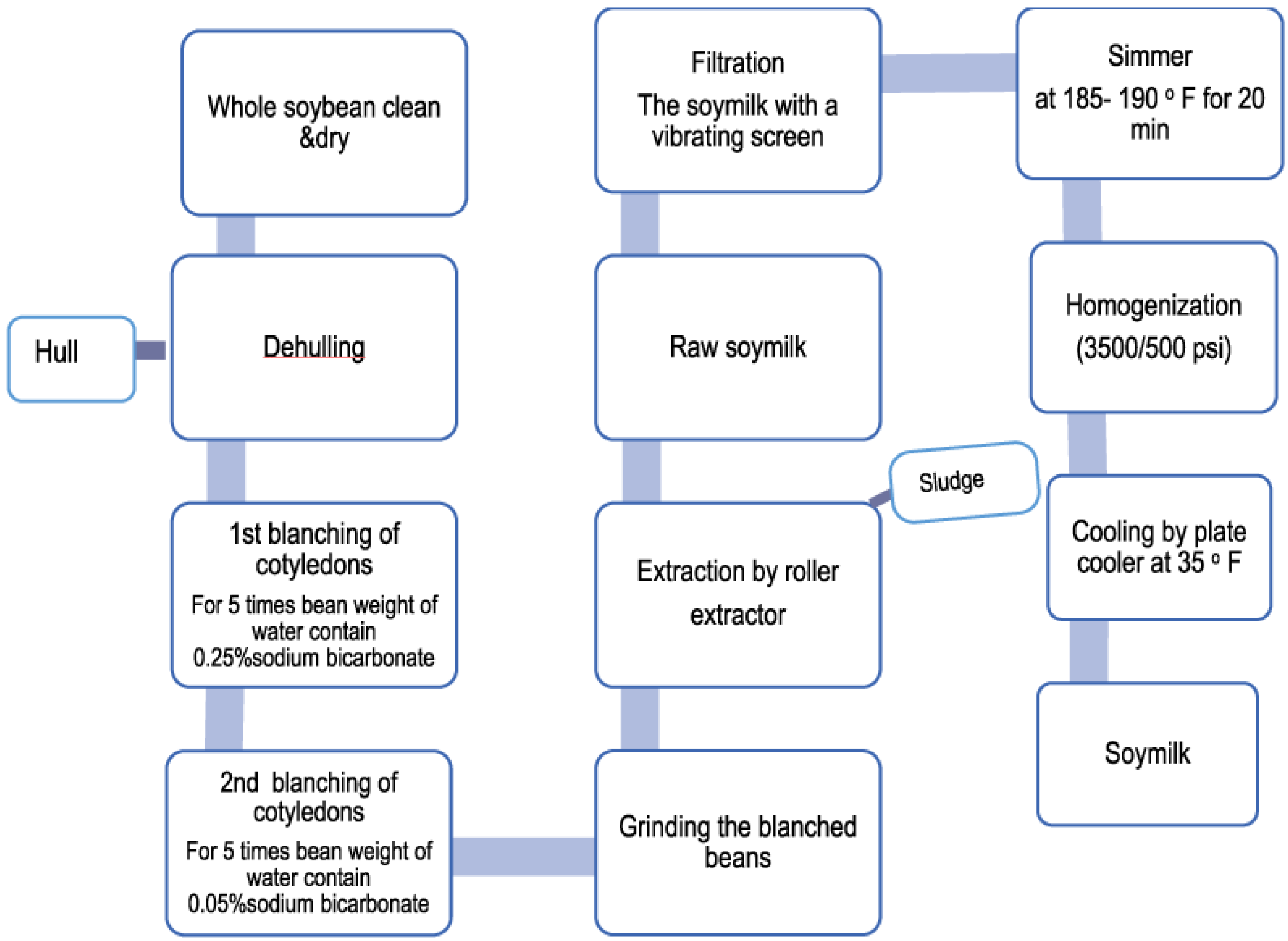

Figure 1: Flow Chart for preparation of soymilk.

Soy-yoghurt manufacture: Plain stirred soy-yoghurt was prepared as reported by EI-Sayed et al. [16]. The method described in Figure 2.

Analytical methods: Moisture, oil, ash and protein (N × 6.25) contents of whole soy bean, cetolydons, hulls and soymilk as well as fiber in soybean seeds & their component were determined according to the AOAC [17]. Soluble sugars were determined by high-performance liquid chromatography (HPLC) as described by Black & Bagley [18] with modifications as follows: Ten ml of 10% lead acetate was added to 25 ml of soymilk to precipitate non carbohydrate compounds from the extract. After centrifugation (10.000 g/15 min) the excess lead acetate was precipitated by 10% oxalic acid, then centrifuged (as above). The supernatant was filtered through a 0.2 µm millipore filter and 1-3 ml was collected in a vial for injection by autosampler in HPLC-equipped with Hewlett-Packard R.I. detector HP 1047 and Bio Rad amnix HPX-87C column (300 × 7.8 mm) and operated at 70°C with degassed and deionizing water as the mobile phase at 0.6 ml/min.

Anti-nutritional factors: The trypsin inhibitors in whole soybeans, cotyledon, and soymilk and soy- yoghurt were measured according to Hamerstand et al. [19]. The phytic acid in whole soybean cotyledon, soymilk and soy-yoghurt was estimated according to the method described by Mohamed et al. [20]. The activity of urease enzyme in whole soybeans, cotyledons, soymilk and soy-yoghurt was estimated according to the technique of AACC [21].

Statistical analysis: The results were analysis statistically using one way analysis of variance (version 16.0 SPSS, Inc., Chicago III, USA). When there was statistically significant difference post hoc comparison were performed with Tukey's test. Values of $P<0.05$ were considered to be significant.

Results and Discussion

Composition of soybean varieties and their seed parts

Major Components: Soybean seeds varieties Clark and Crawford and their structural components (cotyledons and hulls) were analyzed for major chemical composition. Data obtained are presented in Table 1. It could be seen that removing the hulls of both whole soybean

Citation: Abd El-Gawad IA, Hefny AA, Elham El-Sayed M and Saleh FA (2015) Preparation Technique of Soymilk-Based Yoghurt and it's Relation to Soybean Varieties and Anti-Nutritional Factors. J Nutr Food Sci 5: 411. doi:10.4172/2155-96001000411

varieties caused a significantly (p<0.05) decrease in moisture, fiber and carbohydrate and a significantly (p<0.05) increase in protein, oil and ash contents of their cotyledons (Table 1).

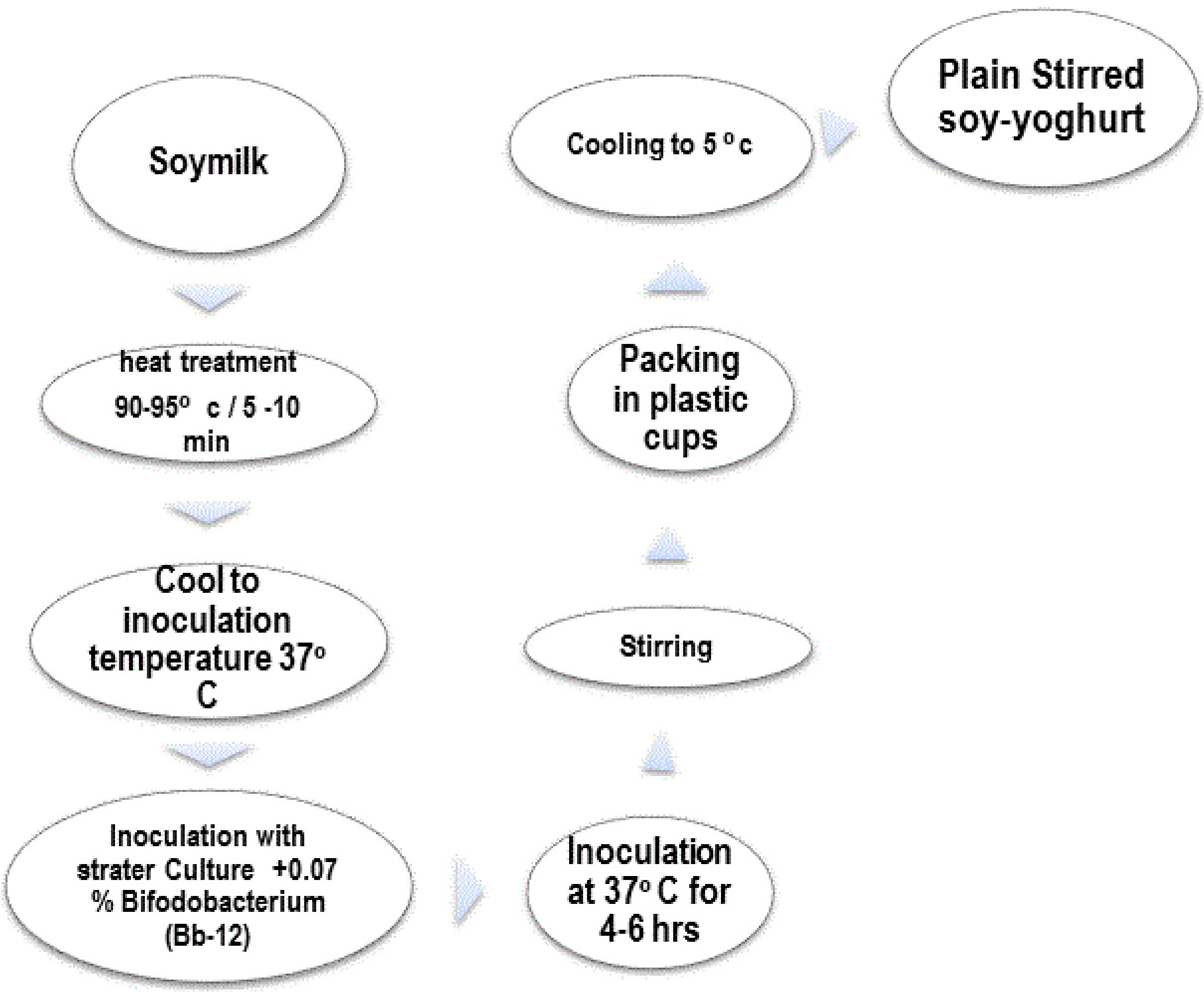

Figure 2: Flow Chart for preparation of stirred soy-yoghurt.

The percentage decrease of moisture, fiber and carbohydrate contents varied from 5.97% to 1.16%, from 3.29% to 30.35% and from 17.63% to 8.56% in Clark and Crawford varieties respectively, whereas the percentage increase of protein, oil and ash varied from 6.14% to 5.75%, from 6.77% to 9.91% and from 17.63% to 8.56% in Clark and Crawford, respectively (Data not shown).

Component %	Soybean varieties					
	Clark			Crawford		
	Whole soybean	Cotyledon	Hull	Whole soybean	Cotyledon	Hull
Moisture	8.37 ± 0.15b	7.87 ± 0.12c	8.61 ± 0.14b	8.61 ± 0.05b	8.52 ± 0.06b	9.26 ± 0.14a
Protein*	37.99 ± 0.27d	40.31 ± 0.16c	12.88 ± 0.12e	41.02 ± 0.14b	43.39 ± 0.41a	9.65 ± 0.17f
Oil*	24.80 ± 0.29b	26.48 ± 0.16a	4.03 ± 0.10d	22.19 ± 0.37c	24.42 ± 0.12b	4.11 ± 0.08d

Ash*	6.79 ± 0.17b	7.20 ± 0.16a	5.50 ± 0.08c	6.87 ± 0.11ab	7.06 ± 0.15ab	5.62 ± 0.16c
Fiber*	6.93 ± 0.22d	6.62 ± 0.12d	49.07 ± 0.16a	10.05 ± 0.13c	7.00 ± 0.11d	42.32 ± 0.32b
Carbohydrates**	23.54 ± 0.14c	19.39 ± 0.10d	28.50 ± 0.10b	19.87 ± 0.18d	18.18 ± 0.11e	38.29 ± 0.38a

Table 1: Chemical composition of two soybean varieties and their seed parts. $^{a-f}$Mean values (n 3) within a row with unlike superscript letters were significantly different (p< 0.05). *Calculated on dry basis. **Calculated by difference=100-(protein+oil+ash+fiber).

As shown in Table 1, the major component in whole beans and cotyledons of Clark and Crawford varieties is protein content, but in hulls of both varieties is fiber content. However, cotyledons of Clark variety contained significantly (p<0.05) lower amounts of moisture (7.87% ± 0.12) and protein (40.31% ± 0.16) and a significantly (p<0.05) higher oil (26.48% ± 0.16) and carbohydrate (19.39% ± 0.10) than those obtained in cotyledons of Crawford variety (8.62% ± 0.05) moisture (41.02% ± 0.14) protein (24.39 ± 0.12) oil and (18.17% ± 0.11) carbohydrate. In this respect, Smith & Circle [22] reported that, there is an inverse relationship between the oil and carbohydrate with the protein.

Soluble sugars: Soluble sugars composition of soybean varieties and their seed parts are presented in Table 2.

Variety/ Sugar	Clark			Crowford		
	Whole	Cotyledon	Hull	whole	Cotyledon	Hull
Stachyose	7.84 ± 0.14^{a}	5.69 ± 0.20^{c}	2.45 ± 0.09^{d}	6.71 ± 0.08^{b}	6.57 ± 0.26^{b}	1.05 ± 0.06^{e}
Rafinose	1.72 ± 0.18^{a}	1.67 ± 0.09^{ab}	0.36 ± 0.08^{d}	1.43 ± 0.06^{bc}	1.21 ± 0.11^{c}	0.34 ± 0.05^{d}
Sucrose	5.14 ± 0.36^{d}	7.39 ± 0.11^{a}	1.15 ± 0.03^{e}	6.04 ± 0.09^{c}	6.81 ± 0.13^{b}	1.36 ± 0.08^{e}
Galactose	0.32 ± 0.10^{bc}	0.29 ± 0.08^{c}	0.39 ± 0.06^{bc}	0.51 ± 0.03^{b}	1.05 ± 0.05^{a}	0.25 ± 0.10^{c}
Total sugars	15.02 ± 0.05^{b}	15.04 ± 0.27^{b}	14.35 ± 0.11^{c}	14.69 ± 0.24^{b}	15.61 ± 0.09^{a}	3.00 ± 0.17^{d}

Table 2: Sugars in soybean seeds parts of varieties Clark and Crawford. $^{a-e}$Mean values (n3) within a row with unlike superscript letters were significantly different (p< 0.05).

Whole soybean: The obtained results in Table 2 indicated that, whole beans Clark variety had significantly (p<0.05) higher stachyose (7.84% ± 0.14),raffinose (1.72% ± 0.18) and non-significantly (p<0.05)higher total soluble sugars (15.02% ± 0.05), but had significantly (p<0.05) lower sucrose (5.14% ± 0.36), galactose (0.32% ± 0.1) than those obtained with whole soybean variety Crawford (6.7% ± 0.08, 1.43% ± 0.06, 6.04% ± 0.09, 0.51% ± 0.03 and 14.69% ± 0.24, respectively).

Soybean cotyledons: It is worthy to note that, cotyledon of Clark variety contained significantly (p<0.05) higher raffinose (1.67% ± 0.09) and sucrose (7.39% ± 0.11) and significantly (p<0.05) lower stachyose (5.69% ± 0.20), galactose (0.29% ± 0.08) and total sugars (15.04% ± 0.27) than the corresponding values obtained with cotyledons of Crawford variety (1.21% ± 0.11, 6.81% ± 0.13, 6.57% ± 0.26, 1.05% ± 0.05 and 15.61 ± 0.09, respectively).

Soybean hulls: The presented results illustrated that, hull of Clark variety contained significantly (p<0.05) higher stachyose (2.45% ± 0.09) and total sugars (14.35% ± 0.11) and non- significantly (p<0.05) higher raffinose (0.36% ± 0.08) and galactose (0.39% ± 0.06), but with non-significantly (p<0.05) lower sucrose (1.15% ± 0.03) than the values obtained with hull of variety Crawford (1.05% ± 0.06, 3.00% ± 0.17, 0.34% ± 0.05, 0.25% ± 0.10 and 1.36% ± 0.08, respectively).

Generally, these variations in gross composition and soluble sugars data in whole soybean, cotyledons and hulls between Clark and Crawford varieties were confirmed by Smith & Circle [22], Snyder & Kwon [9] and Wijerante [10]. They observed that, the chemical composition varied considerably with variety, maturity and growing conditions. From the foregoing results, cotyledons of Clark variety contained the highest total solids and lowest soluble sugars and stachyose, which led to lower flatulence problem, compared to that of Crawford variety. Therefore, Clark variety has been selected and used in this study for soymilk and soy-yoghurt preparations.

Composition of soymilk as affected by the extraction ratios: The ratio of cotyledon to water used in the soymilk process can be varied and this affects the quality and composition of soymilk. The percentage of total-solids, proteins, oil, ash and carbohydrate contents in soymilk prepared from soybean Clark variety at various ratios of cotyledon to water are presented in Table 3. It could be noticed that the soymilk constituents increase as the cotyledon to water ratio changed from 1: 10 to 1:4. Thus, richer soymilk can be prepared by lowering the amount of water in the ratio. The same results have been reported by Chang et al. [14] and Tanteeratarm et al. [15]. However, rate of soymilk production is very much dependent upon the amount of soybeans and water used. Thus, the production rate of a given soymilk system should be reported as a production rate of soymilk of specified protein or solid content.

Dry cotyledons to water ratio	Soymilk Composition %*				
	Total solids	**Protein**	**oil**	**Ash**	**Carbohydrates**
1:04	11.23 ± 0.19	5.19 ± 0.11	2.25 ± 0.10	0.66 ± 0.10	3.13 ± 0.06
1:05	09.69 ± 0.10	4.84 ± 0.08	1.32 ± 0.12	0.41 ± 0.07	3.12 ± 0.06
1:06	07.51 ± 0.19	4.15 ± 0.14	1.26 ± 0.08	0.34 ± 0.05	1.78 ± 0.07
1:07	07.05 ± 0.04	3.80 ± 0.11	0.95 ± 0.06	0.32 ± 0.05	1.99 ± 0.07
1:08	06.86 ± 0.07	3.64 ± 0.10	0.88 ± 0.15	0.28 ± 0.03	2.06 ± 0.09
1:09	06.15 ± 0.12	3.24 ± 0.06	0.86 ± 0.12	0.27 ± 0.03	1.78 ± 0.17
1:10	05.71 ± 0.09	2.95 ± 0.07	0.81 ± 0.10	0.26 ± 0.05	1.70 ± 0.12

Table 3: Composition of soymilk from various dry cotyledons to water ratio. *Mean values (±SD; n=3).

Anti-nutritional factors of soybean, soymilk and soy-yoghurt

Trypsin inhibitors: As shown in Figure 3a Trypsin inhibitors activity (TIA) varied from 46.25 ± 0.35 mg/g whole of Clark soybean to 42.29 ± 0.68 mg /g cotyledon of Clark soybean. This result revealed that, dehulling of whole soybean caused a slight decrease in the trypsin inhibitors activity in cotyledons compared to whole bean cotyledons. Thus, it must be treated to improve their nutritional value. During preparation of soymilk under alkaline conditions (pH 7.1-7.3), blanching of cotyledons (Boil) in the presence of baking soda (0.25% followed by 0.05%) and cooking of soymilk (85-88°C) has an important function, i.e. completely removal of trypsin inhibitors (TI) in soymilk and consequently soy-yoghurt (Figure 3a). The Same result was reported by Lei et al. [12] and Kowk et al. [13]. They found, heating soymilk under alkaline conditions rendered trypsin inhibitors (TI), which are more heat-labile. Heating soymilk under alkaline conditions adopted in this study was more effective on TI than previous reports on heat inactivation of TI in soymilk, which were mostly in the temperature range 93-121°C [11,12]. They found, inactivation of 90% of the native trypsin inhibitors activity (TIA) in soymilk could be achieved by heating for 93°C for 60-70 min. or 121°C at 5-10 min. Ultra high-temperature adopted by Kwok et al. [13] has revealed that, the holding times required to inactivate 90% of the TIA in soymilk at pH 6.5 were 60 min., 56 sec., and 23 sec. when heated at 93°C, 143°C and 154°C, respectively. Rouhana et al. [23] found that, a reduction of TIA in soymilk to about 20% level obtained in batch boiling process requires a heat treatment period, 77 sec. at 140°C. Abu-Salem et al. [24] reported that cooking (boiled in water 3:1 w/v for 30 min.) was the most effective in reducing the activity of trypsin inhibitor.

Urease activity: As shown in Figure 3b, urease activity varied from 1.97 ± 0.12 ΔpH in whole soybeans to 1.99 ± 0.20 ΔpH in cotyledons. This was attributed to removing the hulls, which caused an increase in urease activity in the cotyledons, and that means, the hulls have very low urease activity. Smith & Circle [22] reported similar findings. Soymilk processing under alkaline condition, as above mentioned, have completely destroyed urease activity in soymilk, consequently soy-yoghurt (Figure 3b). These conditions were more effective on urease activity in soymilk than previous study on heat inactivation of urease activity in soymilk, which were mostly in the temperature range of boiling and toasting process [25]. Soybean processing plants are typically using urease activity, ranging from 0.15 to 0.25 Δ pH, as an index to indicate proper heat treatment for animal feeds as well as for trypsin inhibitors and other enzymes inactivation for human diets [9,26]. Although it is not necessary to destroy all the urease activity as obviously mentioned. Our results showed that, urease activity was completely destroyed in soymilk, consequently soy-yoghurt (Figure 3b).

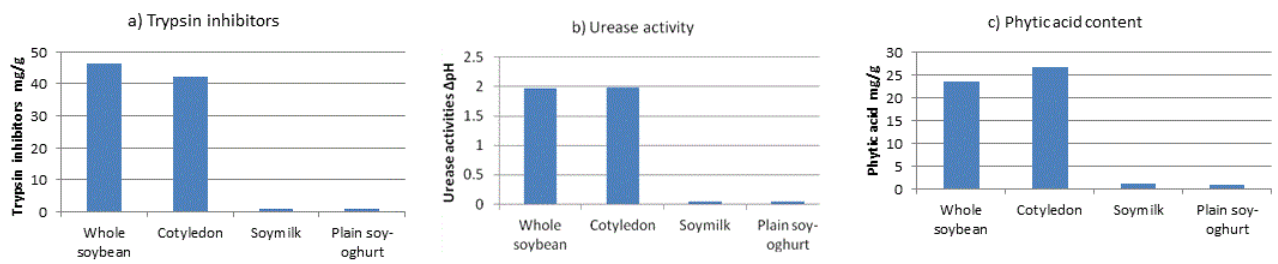

Figure 3: Changes in soybean anti-nutritional agents: Trypsin inhibitors (a), urease activity (b) and phytic acid content (c) affected by preparation technique of soy-yoghurt.

Phytic acid: As shown in Figure 3c phytic acid varied from 23.7 ± 0.31 mg/g Clark whole soybeans to 26.8 ± 0.28 mg/g in their cotyledons. This result revealed that, dehulling of whole soybean caused an increase in the phytic acid of the soybean cotyledons. This could be attributed to the increase of cotyledons proteins (Table 1), which include phytic acid (Lott and Buttrose, 1978). Preparation technique of soymilk and soy-yoghurt in this study, improved the quality of both soymilk and soy-yoghurt, where phytic acid contents in these products were 0.12% (1.15 ± 0.07 mg/g) and 0.11% (1.10 ± 0.12 mg/g) respectively. Kakada et al. [4] and ChurelIa et al. [27] found that no symptoms of decreasing growth or mineral bioavailability to rats, when fed diets containing 0.1 to 0.4% phytic acid. In our results the percentage decrease of phytic acid in soymilk or soy-yoghurt compared to whole soybeans and their cotyledons was 94.94% & 95.52% or 95.36% & 95.90% respectively. These results are in agreement with previously mentioned by Kakada et al [4] and Churella et al. [27]. The decrease in phytic acid content was attributed to removal of water soluble phytic acid in the discard blanch water, and partially to removal of water-soluble and insoluble phytic acid in the separated sludge. Mohamed et al [28] reported that fermentation and germination in combination with the hulling and cooking processes cause significant decrease in phytic acid content more than that other of processing treatments [29,30].

Conclusion

The presented results has demonstrated that the making a survey of available types of soy beans in order to select a high grade beans had a marked effect on the quality of prepared soymilk and soy-yoghurt. Additionally, soymilk and soy-yoghurt produced through this study can furnish high nutritional benefits because trypsin inhibitors and urease activities were completely destroyed, and phytic acid was greatly reduced.

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