Effect of Autoclaving on Chemical, Functional and Morphological Properties of Chickpea (*Cicer arietinum* L.) Starch

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Abstract

Starch is the most abundant carbohydrate in chickpea and it is considered to be competitive in the food industry. The objective of the study is to characterize the native and autoclaved chickpea starch. Starch was isolated, autoclaved and its chemical, functional and morphological properties were analyzed. Native chickpea starch and Autoclaved chickpea starch stored at 4°C and -20°C consisted moisture (8.61± 0.00 %, 7.52± 0.00% and 7.72± 0.00 %), dry matter (91.58± 0.17, 92.39± 0.21 and 92.16± 0.07 %), ash (0.23±0.15%, 0.8± 0.54% and 1.53±0.92%) and amylose (33.19±0.21%, 36.67±0.09% and 38.03±1.07%).Native starch and autoclaved chickpea starch stored at 4°C and -20°C had water absorption capacity (0.9±0.2 ml/g, 4.2±0.19 ml/g and 5.3±0.87 ml/g), oil absorption capacity (0.9±0.01ml/g, 0.73±0.05ml/g and 0.76±0.05 ml/g) and bulk density (0.71±0.02 g/ml, 0.56±0.05 g/ml and 0.34±0.07 g/ml). Swelling power of native chickpea starch and autoclaved chickpea starch stored at 4°Cand -20°C was increased with increase in temperature (50^{o}C to 90^{o}C). Solubility of native chickpea and autoclaved chickpea starches stored at 4°C and -20°C was increased till 70°C then decreased. Microscopy (SEM) analysis revealed that autoclaving altered the granular structure of chickpea native starch. Hence autoclaving could modify the native chickpea starch to obtain unique properties in starch based food products.

KEYWORDS: Native chickpea starch, Autoclaved chickpea starch, Swelling power, SEM

1.Introduction

Chickpea (*Cicer arietinum* L.) is the largest produced food legume in South Asia and the third largest produced food legume globally, grown in more than 50 countries. Asia accounts 89.7% of the area in chickpea production followed by 4.3% in Africa, 2.6% in Oceania, 2.9% in America and 0.4% in Europe (Gaur, 2010). It is the world's third largest pulse crop based on cultivated area (Hasan *et al*. 2008). India ranked first in terms of chickpea production and consumption in the world. About 65% of global area with 68 % of global production of chickpea is contributed by India (Reddy and Mishra, 2010). India contributes 75% of the total world production of chickpea followed by Turkey and Pakistan (Singh *et al*. 2004). Chickpea is a legume of great importance, has an average composition of 16-21% protein, 3% ash, 3-7% lipids, 5-13% crude fibre and 59-67% carbohydrates, and of the total grain carbohydrates, about 40-50% is starch (Singh *et al*. 2004; Costa *et al*. 2006). Carbohydrates constitute the main fraction of cereals, legumes, tubers and unripe fruits, accounting for up to 40–80% of the dry matter (dm) (Skrabanja *et al*. 1999). Starch is the principal carbohydrate in these vegetable sources and it is traditionally isolated from many of them for diverse applications. The current tendency is to look alternative sources for obtaining starches exhibiting better physicochemical and functional characteristics. In recent years, substantial progresses have been made in obtaining starches from these sources, and study of functional and physicochemical properties of starches (Hoover, 2001). Native starches have limited use in food industries. Starch modification will enhance its properties particularly in specific applications such as to improve the increase in water holding capacity, heat resistant behavior, reinforce its binding, minimized syneresis of starch and improved thickening (Adzahan, 2002; Miyazaki *et al*. 2006). There is a huge market for many new functional and added value properties resulting from these modifications. Hence the food manufacturers usually desire starches with better behavioral characteristic than those provided by native starches (Adzahan, 2002). Application of starch in various products and manufacturing processes is primarily determined by its functional properties such as gelatinization, pasting, retro gradation, viscosity, swelling and solubility which vary considerably from crop to crop and with ecological and agronomic influences (Yuan *et al*. 2007). Therefore, understanding the physicochemical and functional properties of starch from different crops could help in utilization of different starch crops for different applications (Mufumbo *et al*. 2011). There is no dearth information on the effect of autoclaving on characteristics of chickpea starches hence the objective of this study was to know the effect of autoclaving on chemical, functional and structural properties of chickpea native starch.

2. Materials and Methods

2.1. Selection of samples and isolation of starch from chickpea

Chickpea seeds were purchased from Tamil Nadu Agricultural University, Coimbatore. The seeds were cleaned to remove unwanted materials such as sand and other impurities. Starch was isolated according to the method of Miao *et al*. (2009). Chickpea seeds were soaked in an excess of deionized water containing 0.2% sodium hydrogen sulphite at 20°C over night and the teaste was removed manually .The decorticated grains were ground in a laboratory blender , filtered through 100-mesh sieve, the filtrate was centrifuged at 3000g for 10min. The sediment was washed repeatedly with distilled water, until the supernatant was clear and the starch was free of colour, collected and dried in an oven at 40°C for 12 h.

2.2. Autoclaving –cooling cycles of chickpea starch

Autoclaving cooling cycles of chickpea starch was done by the method of Berry (1986) with slight modification. The chickpea starch (40g) was mixed with 160ml of water, pressure-cooked at 121°C for 1hr in an autoclave, cooled down at room temperature, part of starch sample was stored at 4°C and the remaining sample at -20°C for 72h. After three autoclaving cooling storage cycle, the sample was freeze-dried and ground into fine particles (100 mesh).

2.3. Properties of chickpea native starch and chickpea autoclaved starches stored at 4°C and -20°C

Moisture content and dry matter were estimated by the method of Adebayo *et al*. (2010). Ash content was estimated by AOAC Method (1990). Amylose content was determined by the method of Williams *et al*. (1958). Water/oil absorption capacity and bulk density was determined according to the method of Abbey and Ibeh (1988) and Okaka and Potter (1977) respectively. Swelling power and solubility of the starches were determined by the method of Gani *et al*. (2010). Granular morphology of chickpea native starch and chickpea autoclaved starches stored at 4°C and -20°C was examined by Scanning Electron Microscope (SEM). Prior to the examination, starch was dried overnight in an air oven at 80°C and mounted on a stub with double sticky tape. The stub was then coated with a thin layer of gold in order to improve conductivity and prevent electron charging on the surface. SEM was operated at 15 kV to image the samples (Aht-Ong and Charoenkongthum, 2002).

3. Statistical analysis

Quantitative data analysis was carried out using MS-Excel 2007. Mean and standard deviation were calculated. All data obtained were subjected to One Way Analysis of Variance (ANOVA) and the means were compared by Critical Difference (CD). Differences at $P<0.05$ were considered to be significant.

4. Results and Discussion

Moisture, dry matter, ash and amylose content of the chickpea native starch, chickpea autoclaved starch stored at 4°C and -20°C is given in the Table 1.

Moisture content of chickpea native starch and chickpea autoclaved starch stored at 4°C and -20°C was 8.61± 0.23%, 7.50± 0.15% and 7.84± 0.05% respectively. This result is confirmed with Huang *et al*. (2007) who stated that the moisture content of native chickpea starch was 11.9%. Dry matter content of chickpea native starch and chickpea autoclaved starch stored at 4°C and -20°C was 91.58± 0.17%, 92.48± 0.15% and 92.15± 0.05% respectively. Ash content of the chickpea native starch was 0.23±0.15% and the chickpea autoclaved starch stored at 4°C and -20°C was 0.89± 0.18% and 1.57±0.16% respectively. Ash content of the chickpea autoclaved starch stored at 4°C and -20°C was significantly ($P<0.05$) higher than chickpea native starch. Similarly Aparicio-Saguilána *et al*. (2005) reported that ash content of the autoclaved banana starch (0.71%) was higher than native banana starch (0.45%). This result is confirmed with Nuwamanya *et al*. (2011) who stated that the ash content of chickpea starch was 0.2%. Amylose content of chickpea native starch, autoclaved starch stored at 4°C and -20°C was 33.19±0.21%, 36.67±0.09% and 38.03±1.07% respectively. This result is confirmed with Sing et al. (2004) who stated that amylose content of different chickpea cultivars is to be in the range of 28.6-34.3 %. Aparicio-Saguilana *et al*. (2005) reported that native banana starch exhibited lower amylose content (37%) than the autoclaved material (44.8%) which is indicative of partial debranching of amylopectin due to the drastic pressure-heating. Legume starches have been characterized by high amylose contents, and the amylose levels of chickpea starches were within the range of 20.7-42.2% (Hoover and Rantnayake, 2002).

Water absorption capacity (WAC) of chickpea native starch, chickpea autoclaved starch stored at 4°C and -20°C was 0.9±0.2ml/g, 4.2±0.19ml/g and 5.3±0.87ml/g respectively. This result is confirmed with Singh *et al*. (2004) who stated that water absorption capacity of chickpea starch was in the range of 77.8-89.4%. The increase in water binding value was mainly due to the gelatinization caused by heating and autoclaving at higher temperature (Dundar and Gocmen, 2013). Oil absorption capacity (OAC) is useful in structure interaction in food especially in flavor retention, improvement of palatability and extension of shelf life particularly in bakery or meat products (Adebewale and Lawal, 2004). Oil absorption capacity of chickpea native starch, chickpea autoclaved starch stored at 4°C and -20°C was 0.9±0.1ml/g, 0.73±0.05ml/g and 0.78±0.02ml/g respectively. This result is on par with Sirivongpaisal (2008) who stated that oil absorption capacity of Bambara groundnut starch was 1.01 g/ml. The mechanism of OAC is mainly due to the physical entrapment of oil by capillary attraction (Kinsella, 1976). However, the hydrophobicity of proteins also play a major role in oil absorption (Voutsinas and Nakai, 1983). Bulk density (BD) of chickpea native starch, chickpea autoclaved starch stored at 4°C and -20°C was 0.71±0.02 g/ml, 0.56±0.05 g/ml, and 0.34±0.02g/ml. This result is in confirmty with Agunbiade and Longe (1999) who reported that bulk density of legume starches was ranged from 1.01-1.08 g/ml. Low bulk density is desirable in preparation of infant and weaning foods (Nicole *et al*. 2010).

Swelling power of chickpea native starch and chickpea autoclaved starches stored at 4ºC and -20ºC for different temperatures ranging from 50 to 90°C is shown in the Figure 1. The swelling power of chickpea native starch and chickpea autoclaved starches stored at 4ºC and -20ºC increased gradually when the temperature raised to 90°C. This result is confirmed with Sung and Stone (2004) who reported that swelling power of chickpea starch from 60-90°C was in the range of 2.9-9.8 g/g respectively. However swelling power of chickpea autoclaved starch was higher up to 70°C than chickpea native starch subsequently it declined at 80°C and 90°C. At 80°C, the value of chickpea native starch was about 2.5 times as high as that at 70°C. It indicating that, only after the temperature reaches the onset gelatinization point does the starch granule undergo rapid swelling (Huang *et al*. 2007). Low swelling power of starches may be attributed to the presence of a large number of crystallites formed by the association between long amylopectin chains. Crystallite formation increases granular stability, thereby reducing the extent of granular swelling (Miao *et al*. 2009). Swelling volume of starch was affected by amylose content and the structure of amylopectin (Sasaki and Matsuki, 1998), level of amylose lipid complexation, total leached amylose and phosphate content. Amylose lipid complexes reduce swelling power, while existence of phosphate groups in starch increases the water binding capacity of starch, hence, the swelling power increased (Zuluaga *et al*. 2007). Much as it was expected that high amylose starches had high swelling powers, it was observed that at high temperature these patterns change where some starches with high amylose had lower swelling powers at higher temperature. This was also observed when waxy, normal and high amylose wheat was compared (Van Hung *et al*. 2007).

Solubility of chickpea native starch and autoclaved starches stored at 4ºc and -20ºc for different temperatures ranging from 50 to 90°C is shown in the Figure 1. Starch solubility of chickpea native starch and chickpea autoclaved starches stored at 4ºC and -20ºC increased slightly from 50°C to 70°C subsequently declined gradually at 80 and 90°C. Higher solubility range was observed in native starch (4.63 - 6.33) than autoclaved starch (2.33- 4.53) in all temperatures. This result is confirmed with Sung and Stone (2004) who reported that solubility of chickpea starch from 60-90°C was in the range of 2.7-1.2 % respectively. In general autoclaved samples presented lower solubility than corresponding raw materials (native and lintenerized samples), this pattern agrees with the higher RS content recorded for the autoclaved samples which produced higher amount of insoluble material, decreased solubility values (Aparicio-Saguilan *et al*. 2005).

Chickpea native starch and chickpea autoclaved starch stored at 4^{o}C and -20^{o}C were observed by SEM and it is given in the Figure 3. The chickpea native starch showed large oval shaped granules and small spherical shaped granules and smooth granular surface of starches with no evidence of any fissures was observed. Similar observations for different chickpea starches have been reported by Miao *et al*. (2009). Autoclaving and cooling cycles have been shown to influence the granule morphology of native chickpea starch. After autoclaving and cooling cycles, the granular structure was disappeared and some granules with little holes on the surface were appeared. Miao *et al*. (2009) also observed the loss of starch granular structure with autoclaving process in both potato and wax corn starch. The treatment of native starch by autoclaving caused the granular structure of starch broken down to form short-chain linear amylose which were aggregated and developed crystalline particles. The samples were stored at 4°C and -20°C at 72 h observed a more compact structure than native starch .This observation might be associated to a higher level of cavities or channels in the matrix of starches stored at the highest temperature in constrast with the topological structure of starches stored at lower temperatures. Gonzalez-Soto *et al*. (2007) reported that the RS-rich powder showed crystallinity due to reorganization of the starch chain by retrogradation. The microstructure of the RS-rich powder had cavities or channels in the matrix and this was affected by the storage temperature.

5. Conclusion

Chickpea contains starch, in order to utilise chick pea starch as a valuable ingredient in food industry it has to be modified. Autoclaving decreased swelling power as well as solubility of native starch, where as it increased the water absorption capacity and amylose content of chickpea starch. Swelling power and solubility of autoclaved starch was inhibited by high amylose content. Consequently chickpea native granule structure was also changed by autoclaving due to the debranching of amylopectin chains. Hence the study disclosed that autoclaving could be used to change technological properties of chickpea starch.

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Table 1- Chemical composition of chickpea native starch and chickpea autoclaved starch stored at 4°C and -20° C in per cent

Samples	**Moisture**	**Dry matter**	**Ash**	**Amylose**
CNS	8.61± 0.23^{a}	91.58± 0.17^{a}	0.23±0.15^{a}	33.19±0.21^{a}
CASS(4°C)	7.50± 0.15^{b}	92.48± 0.15^{b}	0.89± 0.18^{b}	36.67±0.09^{b}
CASS(-20°C)	7.84± 0.05^{c}	92.15± 0.05^{c}	1.57±0.16^{c}	38.03±1.07^{c}

CNS = Chickpea native starch, CASS4°C = Chickpea autoclaved starch stored at 4°C, CASS -20°C = Chickpea autoclaved starch stored at -20°C. All values are means of triplicates ± standard deviation. $^{a-c}$ Means in the same column with different superscripts are significantly different (P<0.05).

Table 2- Functional properties of native chickpea starch and Autoclaved Chickpea Starch Stored at 4°c and -20°c.

Samples	WAC (ml/g)	OAC (ml/g)	Bulk density (g/ml)
CNS	0.9±0.2[a]	0.9±0.1[a]	0.71±0.02[a]
CASS at (4°C)	4.2±0.19[b]	0.73±0.05[b]	0.56±0.05[b]
CASS at (-20°C)	5.3±0.87[c]	0.78±0.02[bc]	0.34±0.02[c]

All values are means of triplicates ± standard deviation. [a-c] Means in the same column with different superscripts are significantly different ($P<0.05$). WAC-Water absorption capacity, OAC-Oil Absorption Capacity. CNS = Chickpea native starch, CASS at 4°C = Chickpea autoclaved starch stored at 4°C, CASS at -20°C = Chickpea autoclaved starch -20°C.

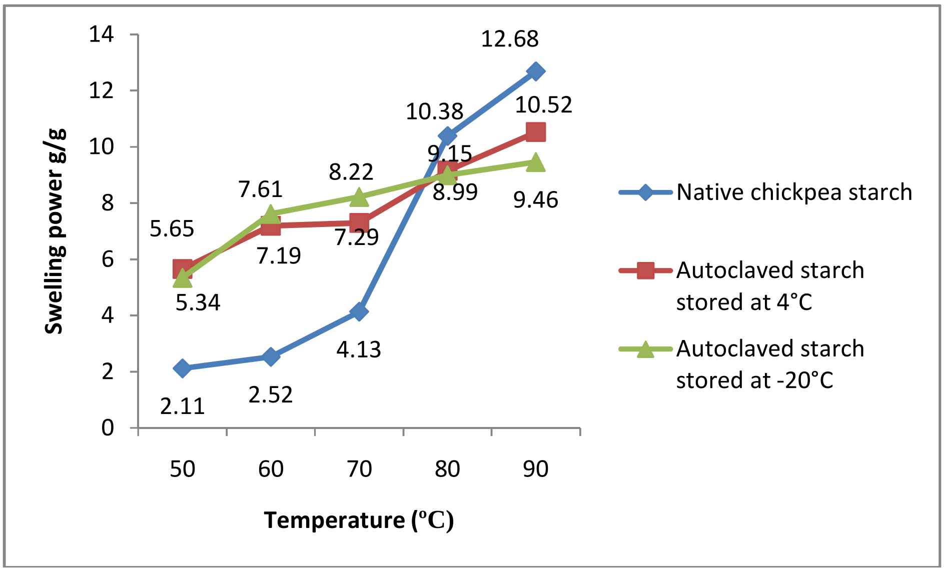


Figure 1 – Swelling power of native chickpea starch and autoclaved chickpea starches stored at 4°C and -20°C submitted to heat from 50 to 90°C.

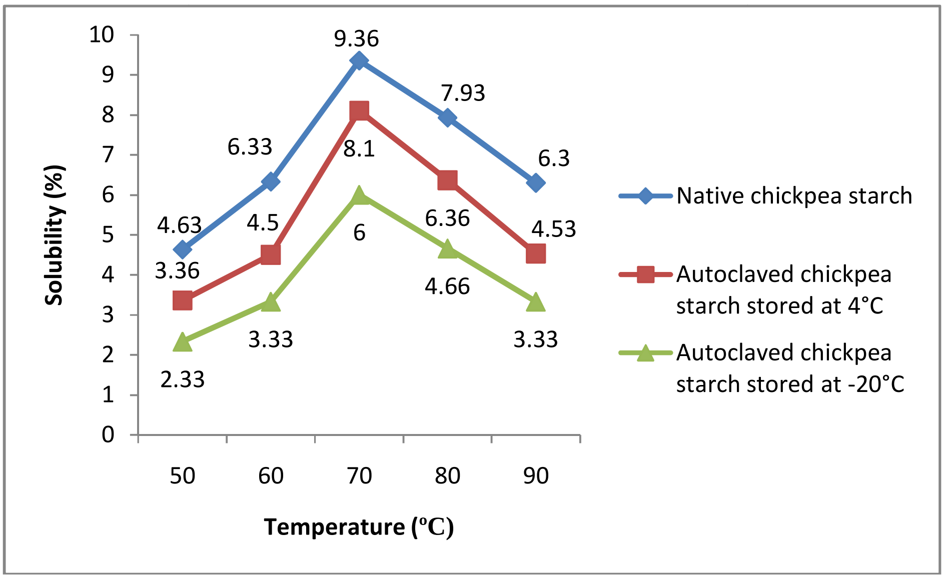
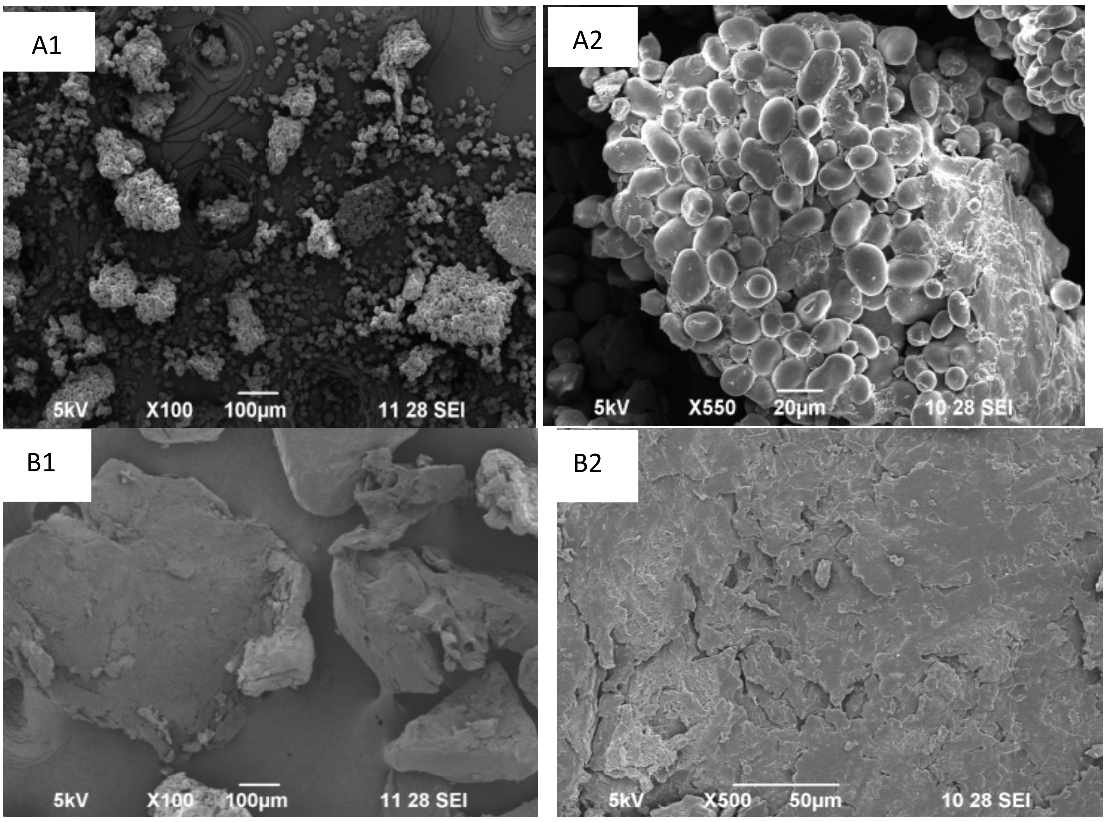
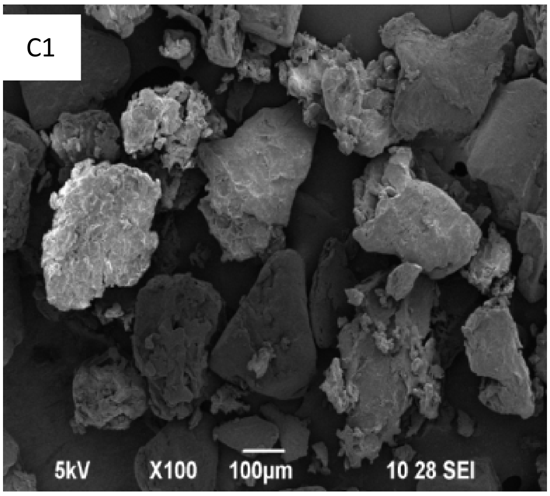


Figure 2 – Solubility of native chickpea starch and autoclaved chickpea starches stored at 4°C and -20°C submitted to heat from 50 to 90°C.





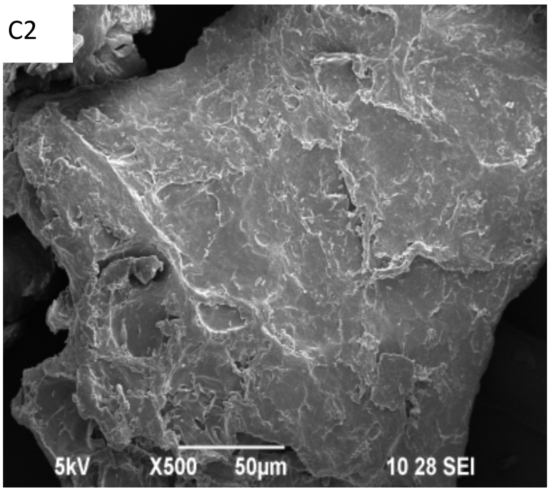


Fig.3. Scanning electron micrograph of chickpea native starch and chickpea autoclaved starch stored at 4ºC and -20ºC.

1. Chickpea native starch (A1); 2. Chickpea autoclaved starch at 4ºC (B1) 3. Chickpea autoclaved starch at -20ºC (C1). (A1)Scanningelectronmicrographs of Chickpea native starch at 100X (magnification) and (A2) 550X (mangnification). (B1)Scanning electron micrographs of Chickpea autoclaved starch at 4^{o}C 100X (magnification) and (B2) 500X (magnification). (C1) Scanning electron micrographs of Chickpea autoclaved starch at -20^{o}C 100X (magnifications) and (C2) 500X (magnification).