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Potravinarstvo® Scientific Journal for Food Industry
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potravinárstvo®

Potravinarstvo, vol. 10, 2016, no. 1, p. 195-201
doi:10.5219/627
Received: 18 April 2016. Accepted: 19 April 2016.
Available online: 13 May 2016 at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECTS OF CROSS-LINKING MODIFICATION WITH PHOSPHORYL CHLORIDE ($POCL_3$) ON PYSIOCHEMICAL PROPERTIES OF BARLEY STARCH

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ABSTRACT

Chemical methods are one of the comon method in starch modification. This study aimed at investigating of cross-link affection of phosphoryl chloride with two different levels 0.5 and 1g.kg^{-1} in order to enhance funciotnal proeprties and physiochemical changes on extracted starch from barley variety Bahman which cultivates in Chahr-Mahal Bakhtiari Province of Iran. Obtained results indicated that cross-linking leads to reduce sweeling power of strach granuls compred to natural starch and the amount of reduciton increase via the substitituin level. Powerfull cross-linkingnetween starch chains casue more resistance of granules to seweeling which is increased by means of cross-linking dgree. Additioally, investigationresults from synersis revealed that releasing water percentage in cross-linked starches increase in comparison to natural starches and this amount depends onthe amount of cross-link surface with a significantly difference in (α <0.05). Gelatinization temperature in both levels negligibly increased by modification where in low level of cross-linking was more. Furthermoe evaluating gelation temperatures of both natural and cross-linked modified starches showed that addition of phosphate groups in starch and creating extra coovalent bonds make granues more compressed reulting in slight increase of To, Tp, Tcin barley starch. Icreasing of temperature observed more in less concentration of cross-links. Evaluation of viscosity changes also revealed that this modification depending on increasing the amount of Phosphoryl Chloride led to increasing peak temperature, diminish peak and setback viscosity. Result also exhibited that in morphological level, cross-link causes to incidence changes in particles' diameter size. The comparison of diameter average and frequency between natural starch and cross-links starch exhibited that in cross-linkd treatment with 0.5% phosphoryl chloride, increase in frequency of granules with diameter of 6 – 10μm and >20 μm obersced. While frequency of granules with diamater size of 2 – 6 μm and 10 – 20 μm has been reduced to 0 which create bigger granules.

Keywords: Barley; starch; modification; cross-linking; physiochemical properties

INTRODUCTION

Barley belongs to Poaceae and Hordeum species **(Sullivan et al., 2013)** uses more in malting, feeding animals, production of starch and ethanol as well **(Myllärinen et al., 1998)**. Starch is composed of two main constituents including amylose and amylopectin (72 – 87%). Starch is used as thickener, stabilizer, and gelling agent in food industries **(Dubois et al., 2001)**, but due to some restricting factors such as low thermal and cutting resistance **(Singh and Singh, 2005)** high tendency to staling and high synersis (**Yosif et al., 2012)** its application has limited in industries; however application can widen through modification **(Singh and Singh, 2005)**. First time, starch modification operated in year 1800 **(Kaur, Singh and Singh, 2006)**. Several targets define for development of functional properties such as strengthening the bonds, increase of thermal resistance, and increase of water binding capacity, emulsion stability and economic benefits **(Light, 1989)**. Cross-linking or intertwined starch is one of the conventional chemical modifications **(Zhao et al., 2012)**. Cross-linking factors include Sodium triphosphate (STM), Epihydrochlorine (EPI), phosphoril chloride (POCl3), and mixture of adipic acid, anhydride acetic and vinyl chloride **(Singh et al., 2007; Zhao et al., 2012)**. In this method, reaction factors react with starch hydroxyl groups **(Miyazaki et al., 2006)** which enhance through covalent or hydrogen bond inter and among granule molecules **(Singh et al., 2007; Ackar et al., 2010)**. Cross-linked starch strengthens versus heat, acid and cutting in comparison with natural starch **(Hung and Morita, 2005; Polnaya et al., 2013; Raina et al., 2007; Xiao et al, 2012)**. The target of this study is to investigate barley starch properties. Base on **Jun et al., (2003)** barley and corn starches use to microencapsulation of volatile compounds of flavor in meat industry **(Abbas et al., 2010)**.

MATERIALS AND METHODS

In present study, starch has extracted from barley Bahman variety which cultivated in Lordegan region, Chaharmahale Bakhtiari province of Iran. Initially 100 g of barley flour weighed with balance model Mark Sartoris AC 120 s, Germany and 0.0001 accuracy, mixed with 500 mL sodium hydroxide solution (0.005 – 0.025 M) and stirred at 25 °C for 30 min. Obtained mixture centrifuged with 1400 g (centrifuge Tehtnika, model 322-A, Slovenia), then sedimentation filter through a screen with mesh size 270 (50 μm). Permeated suspension neutralized with chloridric acid 1 M and recentrifuged, and over layer of starch separated with spatula remained sedimentation

dissolved in the water again and dissolving continues to reach the minimum amount of creamy layer on it (3 times). Final sedimentation dried in oven (model Mark Memmert UNB-400, Germany) at 40 °C for 24 hr **(Lim et al., 1992)**.

PRODUCTION OF CROSSLINKED STARCH

Regarding production of cross-linked starch, **Kaur et al., (2012)** method used. In this method, 15 g of starch weighed with balance model Marksaritus AC 120S, Germany AND 0.0001 accuracy, then mixed with 24ml water and 0.3 g sodium sulfate added to it, pH of obtained mixture(pH meter model Mark metrum 827, Switzerland used to measured pH) adjusted by sodium hydroxide solution (0.5M) at 25 °C. Phosphoryl chloride (0.5 and 1 g per starch kilo) added by micro-syringe and immediately container sealed. pH adjusted by chloridric acid (0.1 M) on 5.5 after 1hr. sedimentation washed by distilled water and filtered by vacuum filter and finally dried in oven Markmemmert model UNB-400, Germany) at 40 °C **(Kaur et al., 2012).**

DETERMINATION OF SWEELING POWER

Lich et al., (1959) method used to determine swelling power. Initially, 0.1 g sample base on dried weight weighed in lidded test tube and 10 mL water added to it. Tubes placed and shook in water bath (Mark hak model SWB-20, Germany and equipped with shaker with constant race) at 95 °C for 30 min, then cooled to ambient temperature and centrifuged in 2500 x g for 10 min. Supernatant accurately removed and tube containing sedimentation reweighed. Regarding equation 1 swelling power measured **(Leach et al., 1959)**.

Equation (1):

SP – Sweeling percent

$$SP = \frac{\textbf{final weight} - \textbf{weight of empty pipe}}{\textbf{starch weight}} \times \mathbf{100}$$

DETERMINATION OF SYNERSIS PERCENT

To determine synersis percent, **Gioti et al., (2006)** method was used. Starch suspension 5% w/w prepared and 30 min mixed in water bath (model Markmemert w3 B10), heated at 90 – 95 °C, and then quickly cooled to ambient temperature in cooling bath. Starch paste placed at 4 °C for 24 hr, centrifuged at 2700 x g for 15 min and measured released water reported as synersis percent **(Jyothi, Moorthy and Rajasekharan, 2006)**.

INVESTIGATION OF VISCOSITY CHANGES

To determine viscosity changes, Initially, a 8% w/w suspension of starch prepared in pH = 5, then viscosity changes measured by viscometer model Brookfield DV III, America in temperature range 40 – 93 °C, keeping at 93 °C and then reduces it from 93 °C to 40 °C **(Das et al., 2010)**. Determination of substitution degree of cross-link

Substitution degree defines as the number of hydroxyl group in each anhydrous glucose having the ability of derivation with replacing groups **(Yosif et al., 2012)**. To investigate of this factor in cross-link starch, measures based on **Ackar et al., (2010)** method, viscosity data and equation 2 **(Hung and Morita, 2005)**.

Equation (2):

DS – Degree of substitution

$$DS = \frac{\textit{viscosity peak of natural starch} - \textit{viscosity peak of modified starch}}{\textit{viscosity peak of natural starch}} \times \mathbf{100}$$

INVESTIGATION OF THERMAL PROPERTIES

To investigate thermal properties of barley starch **Bello-Perez et al., (2010)** method used. Differential scanning calorimeter used to conduct thermal parameters of starch. In this method, 2 mg starch base on dry weight weighed in aluminum container and 7 mL deionized water added to it, then container sealed, and placed in ambient temperature in order to uniformly distribution of water and homogenization of sample . Sample placed in DSC model F3-200 and heated with race of 10 °C.min^{-1} from 20 °C – 120 °C, and automatically present data including Onset (To), peak (Tp), conclusive (Tc) and ΔH **(Bello-Perez, et al., 2010)**.

INVESTIGATION OF MORPHOLOGICAL PROPERTIES

Electronic microscope model Markzayef used take images and **Blupers et al., (2010)** method with a few changes. Samples fix on a conductive stick and cover with a gold layer **(Bello-Perez, et al., 2010)**. Image proplus software used to analyze images.

Statistical analysis

Complete random design and Duncan test using software SPSS ver. 21th used to conduct statistical analysis of data (α <0.05).

RESULTS AND DISCUSSION

DEGREE OF CROSS LINKING

Table 1 shows that increase of cross linking, cross-linking degree increased. Obtained results were in agreement with **Xiao et al., (2012)**. on investigation of different concentration of epi hydrochlorine on rice starch where cross linking degree increased within increase of epihydrochlorine concentration (16) **Co et al., (2010)**. Reported that an increase 0 – 10% of cross-linking concentration on corn starch cross-linking degree increased **(Koo et al., 2010)**.

SWELL POWER

Comparison of obtained results of swelling power of control and modified samples have summarized in Table 2 revealed a significant difference. Creation of cross-links reduces swelling power of granules with respect to natural starch and this reduction increase by the amount of substitution level. These results were in agreement with **Kim and Yoo (2010)** about using POCl3 on sweet potato and **Majzoobi et al., (2012)** in investigation of wheat starch phosphorlization. It is thought that reduction of swelling power attributed to creation of intermolecular bridges by remained phosphorus after cross linking reaction **(Majzoobi et al., 2012)**.

Cross-linking develops hydrogen bonds among granules and restricts swelling during gelatinization **(Kim et al., 2010)** due to high concentration of cross-linking degree in

Table 1 degree of cross-linking related to control starch and modfied starches.

Treatment	Degree of cross-link percent (%)
Control	0
Cross-linked starch (0.5%)	32.30
Cross-linked starch (1%)	46.15

Table 2 the average of sweeling power related to related to control starch and modfied starches.

Control	Cross-linked starch (0.5%)	Cross-linked starch (1%)
$14.21^{b} \pm 0.66$	$10.05^{a} \pm 2.02$	$7.27^{a} \pm 0.27$

Table 3 the amount of synersis in control and cross-linked starches.

Control	Cross-linked starch (0.5%)	Cross-linked starch (1%)
$65.10^{a} \pm 2.90$	$74.80^{b} \pm 2.34$	$78.94^{b} \pm 6.92$

Table 4 viscosity measurement of control and cross-linked starch.

Starch type	Setback viscosity	Breaking viscosity	Peak viscosity	Pasting Temp. (°C)	peak Temp (°C)
Control starch	5.4	0.96	2.6	63.60	69.55
Cross-linked starch (0.5%)	0.16	1.72	1.76	65.38	72.55
Cross-linked starch (1%)	0.08	1.36	1.4	66.98	76.2

Table 5 Affection of modification amount of thermal properties of barley starch.

Treatment	T_0	T_P	T_C	T_C- T_0	H(J/g)Δ
Control starch	59.8	65.4	73.2	13.4	-0.3412
Cross-linked starch (0.5%)	61.8	66.4	73.8	12	-0.2822
Cross-linked starch (1%)	60.7	65.9	73.4	12.7	-0.2403

presence of more concentrations of cross-linking factor **(Kaur et al., 2012)**.

Choi and Ker (2004) believe that cross-linked starch granules have more resistance to time and temperature of heating. Strong links between starch chains leads to increase of granules' resistance to swelling i.e. by increasing cross-linking degree, resistance increases **(Yosif et al., 2012)**.

AFFECTION OF CROSS-LINKING ON SYNERSIS PERCENT

Comparison of data average in Table 3 shows a significant difference between the amount of released water in natural starch and modified starch. The amount of released water in intertwined starch has increased in comparison with natural starch, furthermore increased by increasing of cross-link factor. These results are in agreement with **Mirmoghtadaei et al., (2009)** on oat starch.

VISCOSITY

Table 4 depicts that heating in 40 – 93°C causes to increase of viscosity gradually. When starch heats in high amount of eater, granules swell, some parts of it dissolves and in form of suspension distributed in surrounded medium (continuous phase) and maximum of viscosity occurs in this point. Continuously, due to dispersion of starch molecule when temperature is constant at 93 °C viscosity decreased, then in temperature reduction from 93 °C to 43 °Conce again viscosity increases. It is thought that arrangement of amylose linear chains (those formerly dissolved as a result of heating and keeping in constant temperature) causes to create lots of cross-links within gel forming process **(Bello-Perez et al., 2010)**.

Investigation of obtained results exhibited that viscosity peak in cross-linked starch has reduced while temperature of viscosity peak increased. It is thought that increase of strong intermolecular bonds due to cross-linking process which results in swelling and decrease of viscosity peak. Besides by increasing of the cross-linking surface, viscosity peak showed more reduction and Peak temperature more increase.

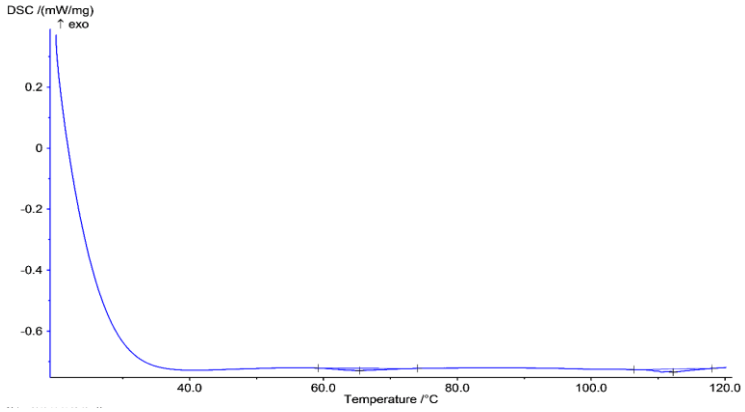

Figure 1 Thermal analysis curve of control starch.

THERMAL PROPERTIES

Analysis of obtained results of Table 5 including analysis of thermal curve related to natural barley starch (Figure 1), curves related to thermal analysis of cross-linked starch with 0.5% (Figure 2) and Figure 3 which shows cross-linked starch with 1% revealed that onset temperature (T0), peak temperature (Tp) and conclusive temperature (Tc) were 59.8 °C, 65.4 °C and 73.2 °C respectively. Obtained temperature were in the range measured by **Gujeral et al., (2013)** where the range of onset, peak and conclusive temperatures were 59.08 – 62 °C, 63.56 – 68.3 °C and 68.56 – 74.71 °C investigated respectively **(Gujral et al., 2013)**. Investigation of natural starch and cross-linked starch in present study suggested that this modification has increased negligibly To, Tp, and Tc of barley starch. These results were in agreement with **Majzoobi et al., (2012)** on wheat starch. Phosphates groups bond with starch molecules through covalent bonds and thus starch granules become more compressed, consequently followed by less molecule motivation and therefore gelatinization occurs in higher temperatures **(Carmona-Garcia et al., 2009)**.

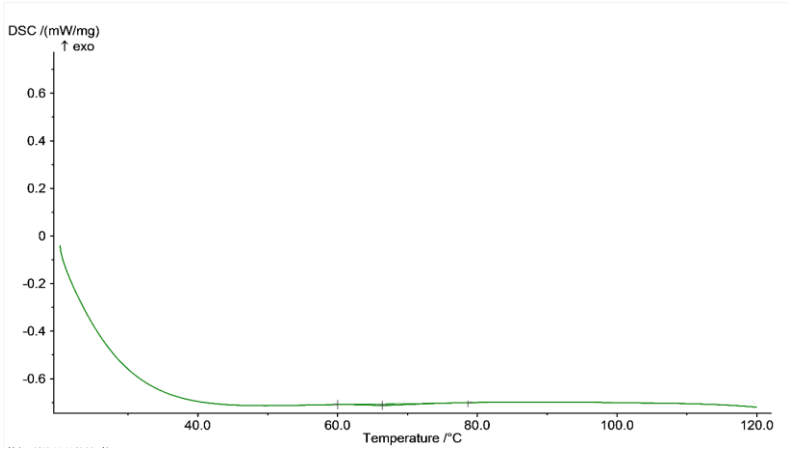

Figure 2 Thermal analysis curve of cross-linked starch (0.5%).

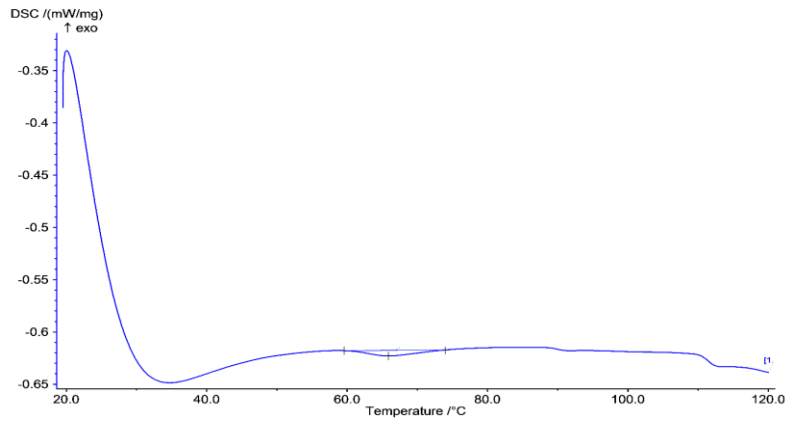

Figure 3 Thermal analysis curve of cross-linked starch (1%).

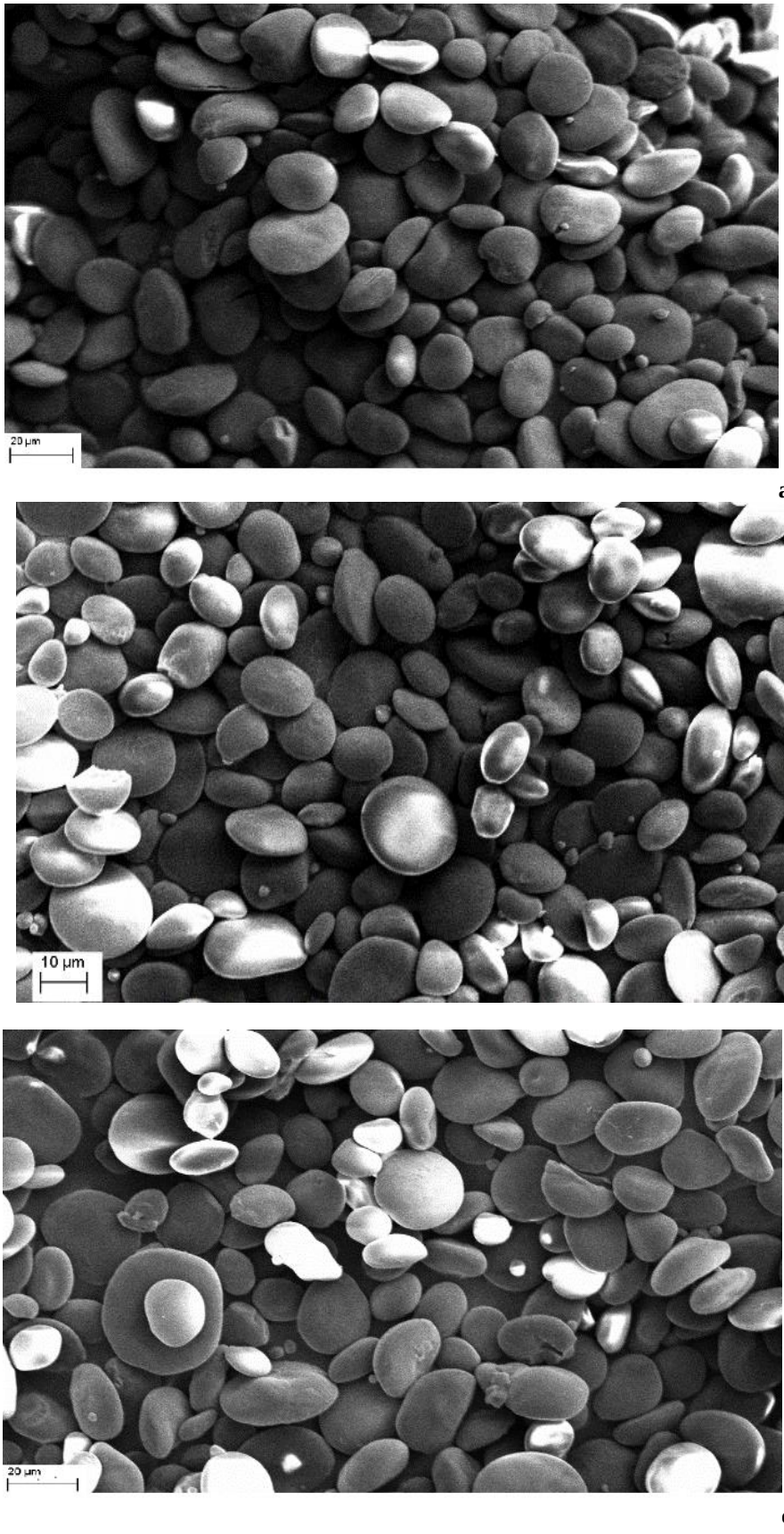

Figure 4 images of electronic microscope: a) control starch b) cross-linked starch (0.5%) c) cross-linked starch (1%) with zoom of 1.5 KX.

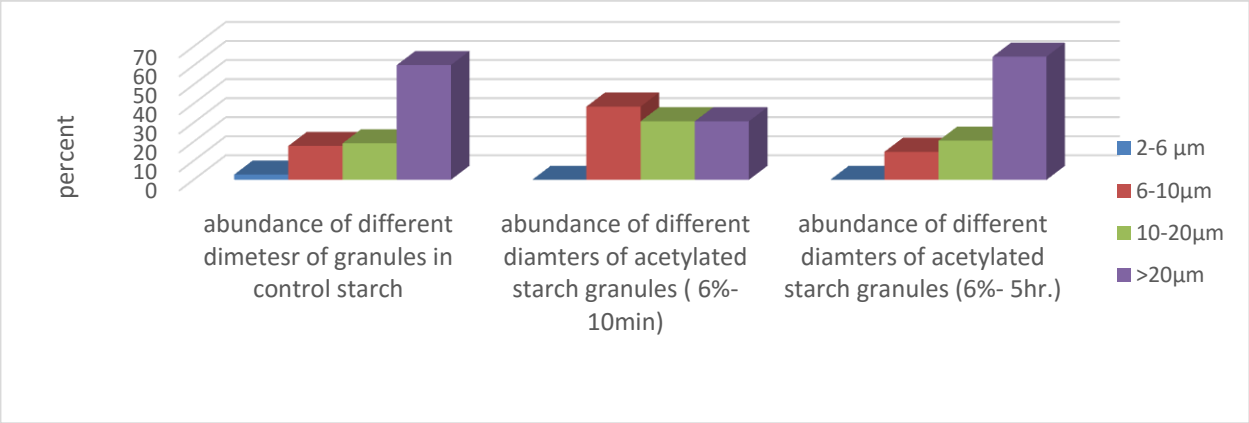

Figure 5 Investigations of abundant percent of granules' thickness in control and cross-linked starches.

Kim and Yoo (2010) found that in sweet potato, cross-linking with POCL3 creates no change in onset and conclusive temperatures. As an appropriate parameter of being crystal, gelatinization enthalpy point at damaging to molecule discipline due to breaking of hydrogen bonds **(Alvani et al., 2011)** after gelatinization. Low enthalpy introduces low stability of crystal structure **(Sing et al., 2006)**.

MORPHOLOGICAL PROEPRTIES

Comparison average of diameter and frequency (Figure 5) between image processing of natural starch and cross-linked starch (Figure 4) suggested that in crossed-link starch with 0.5% phosphoryl chloride, more granules with diameter of 20 µm and 6 – 10 µm observed.

While frequency of granules with diameter of 2 – 6 µm and 10 – 20 µm diminished. It is thought that aggregation of smaller granules and creation of larger granules is the reason of disappear of some size of granules. In cross-linked starch with 1% phosphoryl chloride, more reduction in granules with diameter of 20 µm observed, in addition that frequency of granules with 6 – 20 µm increased.

CONCLUSION

Starch modification creates novel properties in starch. Cross linking causes to increase of synersis and reduce of swelling so that has direct proportional with cross-linking factor (chloride phosphoryl). In comparison with control, Gelatinization temperature of modified starch increased. However no proportional trend observed through increase of cross linking. Furthermore the viscosity of cross-linked starch decreases with respect to control.

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