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Original Contribution

FREEZE-THAW STABILIZATION OF WATER CHESTNUT (*TRAPA BISPINOSA*) STARCH IN THE PRESENCE OF GUMS AND SALTS

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ABSTRACT

Purpose: Four polysaccharides gums (Xanthan gum, Guar gum. Carboxymethyl cellulose and gum Acacia) and salts (Sodium chloride, Potassium chloride, Sodium dihydrogen phosphate and Calcium chloride) were compared for their stabilizing effect in Water Chestnut starch gel against freeze thaw treatments.

Methods: The gums and salts were added in starch at 0.1 – 0.3% and 0.5, 1, & 2% respectively. The starch – gum - salt mixtures were freeze-thawed by storing at -10 °C for 24h and then at 30 °C for 4h.

Results: Among the four gums tested, GG was effective in increasing freeze thaw stability, when 0.2% gum was added, while at 0.3%, gum ACA was more effective than GG. It was noted that the addition of salts increase the stability of the gel towards low temperature. The addition of NaCl at 0.5%, 1%, and 2% showed maximum stability compared to other salts, due to the hydrophilic nature of the sodium chloride enhanced water – holding ability of the starch pastes, thereby limiting amount of water exuded but the reduced stability was observed in the presence of $CaCl_2.2H_2O$.

Conclusion: The addition of salts increased the stability of the mixtures against the freeze thawing at varying concentrations.

Key words: Water Chestnut starch, Gums, Freeze-Thaw.

INTRODUCTION

Starch is a versatile and useful polymer in modifying the textural properties of food, behaving as a thickening and gelling agent. The high carbohydrate content of Water Chestnut makes it powerful source of starch for the industrial and domestic uses. Starches are well known for its properties such as, gelation, gelatinization, pasting, solubility, swelling, paste clarity and freeze thaw stability, which enhances the functional properties of the food products.
Functional properties signify the characteristics that govern the behavior of a food component during processing, storage, and preparation. Any property of the food component, other than its nutritional value, that affects utilization of food is defined as 'functional property' of that food (1, 2). Starches are used as thickening agent (sauces, cream soups, pie fillings), colloidal stabilizers (salad dressings), moisture retainers (cake toppings), gel-forming agents (gum confections), binders (wafers, ice cream cones), and coating and glazing agents (nut meats, candies). The functionality of starch in various foods is dependent on its physicochemical properties (3).

Due to the growing demand of ready-to-eat food items, a range of frozen food products are

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frequently introduced in the markets. However, food product manufacturers have to prepare the food in such a way to lessen the unfavorable effects that freezing and thawing may have. The freezing of foods causes enormous changes to structure, due to a combination of both the formation of ice crystals and the freeze-concentration of solutes and dispersed macromolecules in the unfrozen phase (4). The syneresis and related physical property changes induced by freeze- thaw could be changed by adding a minor amount of hydrocolloids and salts.

In the present study, various polysaccharide gums and salts were compared for their effect in stabilizing a Water Chestnut starch gel.

MATERIAL AND METHODS

Materials:

Dried Water Chestnuts (*Trapa bispinosa*), Food grade Xanthan gum (XG), Guar gum (GG), Carboxy methyl Cellulose (CMC), & Acacia (AC) were purchased from the local market of Karachi, Pakistan. Sucrose, NaCl, KCl, $NaH_2PO_4.2H_2O$ and $CaCl_2.2H_2O$ used were of AR grade.

Methods:

1. Isolation of starch

The starch was isolated by the method of (5,6).

2. Proximate Analysis

Standard Association of Official Analytical Chemistry methods, AOAC (7) were adopted for estimating moisture, ash, crude fiber, protein and fat content.

3. Purification of Water Chestnut starch

Purity (on dry basis), expressed as a total starch percentage was calculated as: 100 – (Crude proteins (N X 6.21) + ash + crude fiber + fatty material). Percent yield was calculated using the equation: (starch weight / grains weight) X 100 (8).

4. Physicochemical properties nWCS, Modified WCS, nWCS-Gums, nWCS – Salt and nWCS-Gums- Salt Mixtures.

Freeze thaw stability of following mixtures was observed according to the proposed methods described below.

4.1. Preparation of Mixtures:

a- **Water Chestnut starch – Gums Mixtures**

1- nWCS (5%) + Gums (0.1%)
2- nWCS (5%) + Gums (0.2%)
3- nWCS (5%) + Gums (0.3%)

b- **Water Chestnut starch – Salt Mixtures**

4- nWCS (5%) + Salts (0.5%)
5- nWCS (5%) + Salts (1%)
6- nWCS (5%) + Salts (2%)

c- **Water Chestnut starch – Gums – Salts Mixtures**

7- nWCS (5%) + Gums (0.1%) + Salt (0.5%)
8- nWCS (5%) + Gums (0.1%) + Salt (1%)
9- nWCS (5%) + Gums (0.1%) + Salt (2%)
10- nWCS (5%) + Gums (0.2%) + Salt (0.5%)
11- nWCS (5%) + Gums (0.2%) + Salt (1%)
12- nWCS (5%) + Gums (0.2%) + Salt (2%)
13- nWCS (5%) + Gums (0.3%) + Salt (0.5%)
14- nWCS (5%) + Gums (0.3%) + Salt (1%)
15- nWCS (5%) + Gums (0.3%) + Salt (2%)

5. Freeze Thaw stability

Freeze thaw stability, a modified method based on (9) was used. 5% starch solution was heated at a temperature of 95 °C with continuous mixing for 20 mins, the paste was poured in plastic cups and frozen at -10 °C for a week. The mixtures were allowed to thaw at 30° C for 4 h and the amount of separated water (FTS, %) from the mixture in the centrifuge tube was reported.

6. Statistical Analysis

The results reported are means of triplicate determinations. The statistical analysis of the results was conducted by the analysis of variance (ANOVA) and LSD test using SPSS version 11.0 for Windows program. Significant differences were reported for $P \leq 0.05$.

RESULT AND DISCUSSION

Water Chestnut starch contained 0.17% of ash, 0.1 % of protein, and 0.19% of fat **(Table 1),** thus giving 99.54 % pure starch. The values are almost similar to the earlier studies on Water Chestnut starch (10). The moisture content was observed 8.5%, the moisture content plays a significant role in the flow and other mechanical properties, although it depends largely on the method, extent of drying and the humidity in the surrounding atmosphere.

Table 1. *Proximate composition of native Water Chestnut starch (nWCS).*

Moisture (%)	8.5
Ash (%)	0.17
Crude Fibre (%)	Nil
Protein (%)	0.1
Fat (%)	0.19

When starch gels are subjected to freeze thaw cycling, the separation of water used in the preparation of gels occur, this is due to the re-association of starch molecules, resulting in the formation of insoluble aggregates. The gels are typified as weepy, grainy, or spongy. The better freeze thaw stability of starch will ultimately enhance its aptness for use in food products. When starch gels were kept at freezing temperature, starch – rich regions were created in the matrix and the water present in the matrix remained partially unfrozen. If the concentration of starch with in the matrix is high, the association of starch chains will form thick filament, whereas water molecules coagulate into ice crystals forming a separate phase. On thawing, ice converts to bulk phase water, which may be readily released from the polymeric network (syneresis). The water thus released leaves the starch gel with sponge like texture (11).

During the present study the gums used with nWCS revealed different results for their freeze thaw stability **(Table 2).** Among the four gums tested, GG was effective in increasing freeze thaw stability, when 0.2% gum was added, while at 0.3%, gum ACA was more effective than GG. It has been reported that GG was effective in synergistic combination with starch in improving freeze thaw stability in frozen pie fillings (12).

Table 2. *Effect of gums addition at concentration of (0.1%), (0.2%) and (0.3%) on (%) freeze thaw stability of nWCS.*

	Concentration of Gums		
nWCS-Gums mixtures	**0.10%**	**0.20%**	**0.30%**
nWCS (without gums)	**37.8**^{a}	**37.8**^{a}	**37.8**^{a}
nWCS + XG	**39.0**^{a}	**37.2**^{a}	**37.0**^{a}
nWCS + GG	**52.0**^{a}	**58.2**^{b}	**36.3**^{a}
nWCS + CMC	**40.7**^{a}	**44.2**^{ac}	**42.0**^{a}
nWCS + ACA	**52.0**^{a}	**46.8**^{c}	**58.5**^{b}

Assays were performed in triplicates. Mean values followed

The effect on freeze thaw stability of nWCS in the presence of salts was also examined. It was noted that the addition of salts increase the stability of the gel towards low temperature **(Table 3).** The addition of NaCl at 0.5%, 1%, and 2% showed maximum stability compared to other salts, due to the hydrophilic nature of the sodium chloride enhanced water – holding ability of the starch pastes, thereby limiting amount of water exuded but the reduced stability was observed in the presence of $CaCl_2.2H_2O$

The effect of salts on nWCS-Gums mixtures is shown in **Tables 4 – 15**. The addition of salts increased the stability of the mixtures against the freeze thawing at varying concentrations of salts. In case of 0.1% XG the increase was noticed at 0.5% concentration of salts. As the concentration of gum increased the increase in freeze thaw stability was shifted to the higher concentration of salts as well. The same pattern of salts was observed in presence of all gums. The most prominent increase in freeze - thaw stability was observed by the addition of NaCl and $NaH_2PO_4.2H_2O$.

Table. 3. *Effect of salts addition at concentration of (0.5%), (1%) and (2%) on (%) freeze thaw stability of nWCS.*

nWCS-Salts mixtures	Concentration of Salts 0.5%	1%	2%
nWCS (without salts)	37.8^{a}	37.8^{a}	37.8^{a}
nWCS + S-1	69.7^{b}	71.0^{b}	64.2^{b}
nWCS + S-2	57.0^{cd}	51.0^{c}	54.0^{c}
nWCS + S-3	60.8^{c}	57.8^{d}	54.0^{c}
nWCS + S-4	50.5^{d}	37.0^{a}	34.7^{a}

Assays were performed in triplicates. Mean values followed by different superscripts with in the same column are significantly different (*P ≤ 0.05).

Table 4. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.1% XG).*

		Concentration of Salts		
nWCS + 0.1% XG + Salts mixtures	Control*	0.5%	1%	2%
nWCS + 0.1% XG + S-1	39.0^{a}	47.7^{b}	21.2^{c}	23.5^{d}
nWCS + 0.1% XG + S-2	39.0^{a}	27.3^{b}	19.7^{c}	22.7^{d}
nWCS + 0.1% XG + S-3	39.0^{a}	46.7^{b}	22.7^{c}	23.2^{c}
nWCS + 0.1% XG + S-4	39.0^{a}	25.7^{b}	29.7^{c}	29.7^{c}

* (nWCS + 0.1% XG)

Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 5. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.2% XG).*

		Concentration of Salts		
nWCS + 0.2% XG + Salts mixtures	Control*	0.5%	1%	2%
nWCS + 0.2% XG + S-1	37.2^{a}	23.0^{b}	46.3^{a}	17.8^{b}
nWCS + 0.2% XG + S-2	37.2^{a}	23.3^{b}	20.3^{b}	11.5^{c}
nWCS + 0.2% XG + S-3	37.2^{a}	15.3^{b}	53.3^{c}	28.3^{ab}
nWCS + 0.2% XG + S-4	37.2^{a}	28.3^{b}	42.7^{c}	29.5^{b}

*** (nWCS + 0.2% XG)**

Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 6. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.3% XG).*

		Concentration of Salts		
nWCS + 0.3% XG + Salts mixtures	Control*	0.5%	1%	2%
nWCS + 0.3% XG + S-1	37.0^{a}	13.0^{b}	22.8^{c}	62.0^{d}
nWCS + 0.3% XG + S-2	37.0^{a}	17.0^{b}	13.3^{b}	22.3^{b}
nWCS + 0.3% XG + S-3	37.0^{a}	24.3^{ab}	17.7^{b}	61.0^{c}
nWCS + 0.3% XG + S-4	37.0^{a}	26.3^{b}	30.5^{c}	39.7^{d}

* (nWCS + 0.3% XG)

Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 7. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.1% GG).*

	Concentration of Salts			
nWCS + 0.1% GG + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.1% GG + S-1	**52.0**[a]	**47.2**[b]	**23.5**[c]	**24.8**[c]
nWCS + 0.1% GG + S-2	**52.0**[a]	**56.2**[a]	**27.8**[b]	**26.8**[b]
nWCS + 0.1% GG + S-3	**52.0**[a]	**43.7**[a]	**31.0**[b]	**23.7**[b]
nWCS + 0.1% GG + S-4	**52.0**[a]	**56.0**[a]	**32.7**[b]	**27.5**[b]

*** (nWCS + 0.1% GG)**
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 8. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.2% GG).*

	Concentration of Salts			
nWCS + 0.2% GG + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.2% GG + S-1	**58.2**[a]	**31.5**[b]	**49.3**[c]	**29.5**[b]
nWCS + 0.2% GG + S-2	**58.2**[a]	**31.7**[b]	**59.7**[a]	**30.5**[b]
nWCS + 0.2% GG + S-3	**58.2**[a]	**28.5**[b]	**34.0**[c]	**33.2**[c]
nWCS + 0.2% GG + S-4	**58.2**[a]	**37.5**[b]	**28.7**[c]	**31.5**[c]

*** (nWCS + 0.2% GG)**
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 9. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.3% GG).*

	Concentration of Salts			
nWCS + 0.3% GG + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.3% GG + S-1	**36.3**[a]	**28.8**[b]	**35.3**[a]	**21.5**[c]
nWCS + 0.3% GG + S-2	**36.3**[a]	**31.3**[a]	**35.3**[a]	**20.3**[b]
nWCS + 0.3% GG + S-3	**36.3**[a]	**34.0**[ab]	**38.5**[ac]	**27.8**[d]
nWCS + 0.3% GG + S-4	**36.3**[a]	**28.3**[b]	**36.0**[a]	**32.2**[c]

*** (nWCS + 0.3% GG)**
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 10. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.1% CMC).*

	Concentration of Salts			
nWCS + 0.1% CMC + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.1% CMC + S-1	**40.7**[a]	**42.7**[a]	**24.5**[b]	**8.3**[c]
nWCS + 0.1% CMC + S-2	**40.7**[a]	**45.3**[a]	**17.2**[b]	**24.8**[b]
nWCS + 0.1% CMC + S-3	**40.7**[a]	**58.3**[b]	**20.5**[c]	**32.8**[a]
nWCS + 0.1% CMC + S-4	**40.7**[a]	**52.0**[ab]	**32.3**[ac]	**32.7**[ac]

*** (nWCS + 0.1% CMC)**
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 11. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.2% CMC).*

	Concentration of Salts			
nWCS + 0.2% CMC + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.2% CMC + S-1	**44.2[a]**	**28.0[b]**	**43.2[a]**	**27.7[b]**
nWCS + 0.2% CMC + S-2	**44.2[a]**	**31.8[b]**	**40.3[a]**	**31.5[b]**
nWCS + 0.2% CMC + S-3	**44.2[a]**	**24.0[b]**	**51.8[c]**	**27.0[b]**
nWCS + 0.2% CMC + S-4	**44.2[a]**	**32.8[b]**	**57.3[c]**	**36.3[ab]**

* (nWCS + 0.2% CMC)
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table. 12. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.3% CMC).*

	Concentration of Salts			
nWCS + 0.3% CMC + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.3% CMC + S-1	**42.0[a]**	**10.2[b]**	**29.8[c]**	**27.3[c]**
nWCS + 0.3% CMC + S-2	**42.0[a]**	**0.8[b]**	**30.3[c]**	**27.3[d]**
nWCS + 0.3% CMC + S-3	**42.0[a]**	**2.0[b]**	**29.2[c]**	**43.8[a]**
nWCS + 0.3% CMC + S-4	**42.0[a]**	**29.7[b]**	**32.2[b]**	**53.3[c]**

* (nWCS + 0.3% CMC)
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 13. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.1% ACA).*

	Concentration of Salts			
nWCS + 0.1% ACA + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.1% ACA + S-1	**52.0[a]**	**43.2[b]**	**24.3[c]**	**33.3[d]**
nWCS + 0.1% ACA + S-2	**52.0[a]**	**55.5[b]**	**24.7[c]**	**28.3[d]**
nWCS + 0.1% ACA + S-3	**52.0[a]**	**55.7[a]**	**29.0[b]**	**30.0[b]**
nWCS + 0.1% ACA + S-4	**52.0[a]**	**44.7[b]**	**29.2[c]**	**29.0[c]**

* (nWCS + 0.1% ACA)
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 14. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.2% ACA).*

	Concentration of Salts			
nWCS + 0.2% ACA + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.2% ACA + S-1	**46.8[a]**	**17.3[b]**	**32.0[c]**	**8.2[b]**
nWCS + 0.2% ACA + S-2	**46.8[a]**	**25.0[b]**	**55.7[c]**	**30.5[b]**
nWCS + 0.2% ACA + S-3	**46.8[a]**	**31.0[b]**	**50.8[a]**	**31.8[b]**
nWCS + 0.2% ACA + S-4	**46.8[a]**	**6.0[b]**	**46.3[a]**	**4.2[b]**

* (nWCS + 0.2% ACA)
Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*P ≤ 0.05).

Table 15. *Effect of salts addition at concentration of 0.5%, 1% and 2% on (%) Freeze thaw stability of (nWCS + 0.3% ACA).*

	Concentration of Salts			
nWCS + 0.3% ACA + Salts mixtures	**Control***	**0.5%**	**1%**	**2%**
nWCS + 0.3% ACA + S-1	**58.5a**	**8.0^{b}**	**29.3^{c}**	**46.3^{d}**
nWCS + 0.3% ACA + S-2	**58.5^{a}**	**27.5^{b}**	**24.2^{b}**	**42.5^{c}**
nWCS + 0.3% ACA + S-3	**58.5^{a}**	**31.5^{b}**	**28.7^{c}**	**52.7^{d}**
nWCS + 0.3% ACA + S-4	**58.5^{a}**	**28.3^{b}**	**34.5^{c}**	**58.3^{a}**

* (nWCS + 0.3% ACA)

Assays were performed in triplicates. Mean values followed by different superscripts with in the same rows are significantly different (*$P \leq 0.05$).

CONCLUSION

Having higher freeze thaw stability of some mixtures, the Water Chestnut starch can easily be used in the preparation of frozen products and other industrial applications. For most applications, however, starches that are even more stable than waxy starches are needed, and such stability is achieved by chemical crosslinking and stabilization. From a consumer perspective and an environmental view, it would be advantageous if freeze- stability could be engineered without the use of such chemical treatments. The use of Water Chestnut starch – gums mixtures will made it use more applicable in food products. This can make this native starch from non-conventional source an excellent alternative to many other conventional starches.

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