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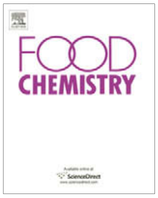

# Effects of cross-linking and acetylation on oat starch properties

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ABSTRACT

Starch samples separated from oat were modified with two different levels of $POCl_3$ (0.5 and 1.0 g $kg^{-1}$) as a cross-linking agent and two different levels of acetic anhydride (6% and 8% (w/w)) for acetylation. Swelling factor, thermal properties and retrogradation measurements were evaluated to characterise the influence of phosphorylation and acetylation on oat starch. Cross-linking decreased the swelling factor and did not improve gelatinization temperature while it increased synaeresis in comparison with native starch. Acetylation increased swelling factor but reduced gelatinization temperature and synaeresis of oat starch.

## 1. Introduction

The interest in oat has been accompanied by an increased interest in study of its starch. Limited research suggests that oat starch does possess some unique chemical, physical, and structural properties (Sowa & White, 1992). Starch plays an important role in textures of many kinds of food products and serves as a major source of energy for humans. In some cases, however, native starch does not meet the functional properties required in food products such as thickening and stabilization. Therefore, starch used in the food industry is often modified to overcome undesirable changes in product texture and appearance caused by retrogradation or breakdown of starch during processing and storage (Van Hung & Morita, 2005).

Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds that act as a bridge between the starch molecules (Jyothi, Moorthy, & Rajasekharan, 2006). Important factors in the cross-linking reaction include chemical composition of reagent, reagent concentration, pH, reaction time and temperature. Because the degree of cross-linking for food starch is very low, the extent of reaction and yield of cross-linked starch are difficult to measure chemically; hence there is a need for physical property measurement. When phosphorus oxy chloride (phosphoryl chloride, $POCl_3$, MW153.3) is added to starch slurry under alkaline conditions (pH 8–12), the hydrophilic phosphorus group immediately reacts with the starch hydroxyls, forming a distarch phosphate (Hirsch & Kokini, 2002).

Cross-linking alters, not only the physical properties, but also the thermal transition characteristics of starch, although the effect of cross-linking depends on the botanical source of the starch and the cross-linking agent. Decrease in retrogradation rate and increase in gelatinization temperature have been observed with cross-linked starch, and these phenomena are related to the reduced mobility of amorphous chains in the starch granule as a result of intermolecular bridges (Singh, Kaur, & McCarthy, 2007). However, Jyothi et al. (2006) showed that cross-linked starch has more pronounced synaeresis than has native starch because of ordered structure in the starch paste, thus resulting in a higher degree of retrogradation.

Acetylation of starch is an important substitution method that has been applied to starch that imparts the thickening needed in food application. Acetylated starch is a granular starch ester with the $CH_3CO$ group introduced at low temperature. Acetylated starch has improved properties over its native form and has been used for its stability and resistance to retrogradation (Singh, Chawla, & Singh, 2004). It increases viscosity, solubility, swelling factor, hardness, cohesiveness, adhesiveness and translucency of the gels while it decreases initial gelatinization temperature (González & Perez, 2002).

Currently, there is little information on the properties of chemically modified oat starch that can be used to develop further applications. Hence, the objective of this study was to examine the influence of two levels of $POCl_3$ and acetic anhydride on physicochemical and thermal properties of oat starch.

## 2. Materials and methods

### 2.1. Material

Oats were grown in the Lavark experimental field of Isfahan University of Technology. The seeds were dehulled and then ground in a disk mill.

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### 2.2. Isolation of starch at alkaline pH

Oat flour (100 g) was mixed with 500 ml of 0.02 M sodium hydroxide, stirred for 30 min at 25 °C, and then centrifuged (1400*g*). The supernatant was discarded, the sediment was slurried with water (500 ml), and the mixture was filtered through a nylon bolting cloth (50 μm). The filtrate was neutralised with 1 M hydrochloric acid, and the mixture was centrifuged. The supernatant was discarded, along with the tailings layered on top of the starch, which were carefully removed by scraping. The starch was washed with water (3 × 200 ml), collected by centrifugation, and dried overnight in a forced-convection oven at 40 °C (Lim, Liang, Seib, & Rao, 1992).

### 2.3. Preparation of cross-linked oat starch

Cross-linking of oat starch was carried out using two different concentrations of $POCl_3$. For this treatment, starch (15 g, dry basis) was suspended in distiled water (24 ml) containing 0.3 g of $Na_2SO_4$ with mild stirring, and then pH was adjusted to 11.5 with 0.5 M sodium hydroxide. The temperature of the slurry was maintained at 25 °C. Phosphoryl chloride was added at 0.5 and 1 g $kg^{-1}$ levels (based on dry weight of starch) using a micropipette and the reaction vessel was sealed. Starch was reacted with $POCl_3$ for 1 h with stirring. After the designated cross-linking time had elapsed, the starch slurry was neutralised to pH 5.5 with diluted HCl (0.1 M). After sedimentation, the starch was washed free of acid, three times, with distiled water, and oven-dried at 40 °C (Kaur, Singh, & Singh, 2006).

### 2.4. Preparation of acetylated oat starch

The method described by Sodhi and Singh (2005) was used to prepare acetylated starch. Starch (100 g) was dispersed in distiled water (225 ml) and stirred for 1 h at 25 °C. Sodium hydroxide (3%) solution was used to adjust the suspension pH to 8.0. Acetic anhydride (6 g) was added drop-wise to the stirred slurry, while maintaining the pH with in the range 8.0–8.4 using 3% NaOH solution. This reaction was allowed to proceed for 10 min after the completion of acetic anhydride addition. The slurry was then adjusted to pH 4.5 with 0.5 M HCl. After sedimentation, It was washed free of acid, twice with distiled water and once with 95% ethanol, and then oven-dried at 40 °C.

### 2.5. Chemical composition of starch

Quantitative estimations of moisture, ash and nitrogen were performed by the standard AACC (2003) methods. Starch lipids were determined by the procedure of Hoover and Vasanthan (1992).

### 2.6. Amylose content

Starch samples (20 mg) were weighed into 100 ml beakers. Exactly 10 ml of 0.5 M KOH solution (28.055 g $l^{-1}$) were added, and the starch was dispersed with a magnetic stirrer for 5 min. The dispersed samples were transferred to 100 ml volumetric flasks and diluted to the mark with distiled water, with careful rinsing of the beaker. An aliquot of the test starch solution (10 ml) was pipetted into 50 ml volumetric flasks, and 5 ml of 0.1 M HCl were added, followed by 5.0 ml of iodine reagent (20 g potassium iodide were weighed into a 100 ml beaker together with 2.0 g of resublimed iodine. The reagent were dissolved in the minimum of water and carefully diluted to 100 ml in a volumetric flask. Then, 10 ml of solution were pipetted into a volumetric flask and diluted to 100 ml with distiled water). The solution was diluted to 50 ml and the absorbance of the blue colour was measured at 625 nm after 5 min. The colour was found to be stable for several hours (Williams, Kuzina, & Hlynka, 1970).

### 2.7. Acetyl percentage and degree of substitution

The percentage of acetylation (% acetyl) and degree of substitution (DS) were determined titrimetrically, following the method of (Sodhi & Singh, 2005). Acetylated starch (1.0 g) was placed in a 250 ml flask and 50 ml of 75% ethanol in distiled water were added. The loosely stopper flask was agitated, warmed to 50 °C for 30 min, cooled and 40 ml of 0.5 M KOH were added. The excess alkali was back-titrated with 0.5 M HCl using phenolphthalein as an indicator. The solution was stood for 2 h, and then the alkali leached from the sample was titrated. A blank, using the original unmodified starch, was also used.

$$\text{Acetyl }(\%) = \frac{[(\text{Blank (ml)} - \text{Sample (ml)}) \times \text{Molarity of HCl} \times 0.043 \times 100]}{\text{Sample weight (g)}} \quad (1)$$

Degree of substitution is defined as the average number of sites per glucose unit that possess a substituent group.

$$\text{DS} = \frac{(162 \times \text{Acetyl }(\%))}{[4300 - (42 \times \text{Acetyl }(\%))]} \quad (2)$$

### 2.8. Swelling factor

Swelling factor and solubility of the starch were determined in triplicate using method of Leach, McCowen, and Schoch (1959) with slight modification. A starch suspension with concentration of 10 g $l^{-1}$ (w/v) was first prepared, and then was heated at 90 °C for 30 min.

### 2.9. Thermal properties

The thermal behaviour of the starch was monitored using differential scanning calorimetry (Mettler TA 4000, Switzerland). Approximately 4.0 mg (dwb) of starch were weighed accurately into an aluminium sample pan. Up to 8.0 mg of distiled water were added, and the pan were hermetically sealed and allowed to equilibrate for 1 h before analysis. Samples were heated at a rate of 10 °C/min from 30 to 120 °C. Onset temperature ($T_0$), peak temperature ($T_p$), conclusion temperature and enthalpy of transition ($\Delta H$) were computed automatically (Wang & White, 1994).

### 2.10. Synaeresis

Starch suspension (5% (w/v)) was heated at 90 °C for 30 min in a temperature-controlled water bath, followed by rapid cooling in an ice-water bath to room temperature. The starch sample was stored for 1–4 and 7 days at 4 °C. Synaeresis was measured as the amount of water released after centrifugation at 3000*g* for 15 min (Sodhi & Singh, 2005).

### 2.11. Morphological properties

Scanning electron micrographs were obtained with a scanning electron microscope (Philips XL30, Netherlands). Starch samples were suspended in ethanol to obtain a 1% suspension. One drop of starch–ethanol solution was applied on an aluminium stub, and left to dry, then coated with gold–palladium (S1508 Sputter Coater, Edwards High Vacuum, West Sussex, UK). An acceleration potential of 10 kV was used during micrography (Sodhi & Singh, 2005).

### 2.12. Statistical analysis

The SAS Software (version 9.1) was used to calculate significant differences in treatment means and LSD ($p < 0.05$) and all measurements were performed in triplicates.

## 3. Results and discussion

### 3.1. Chemical analysis

The results of the proximate analysis of oat starch are presented in Table 1. The protein content of oat starch was 0.28%, moisture 8.28%, ash 0.33%, fat 0.5% and amylose 26.7%. Such a small amount of nitrogen contributed to endosperm storage proteins, lysophospholipids and proteins located inside starch granules (Hoover, Smith, Zhou, & Ratnayake, 2003). It is well documented that protein contamination of starch with up to 5% of protein had no significant effect on thermal properties of starch. Thus, this starch might be considered as sufficiently pure for further analysis (Sowa & White, 1992).

**Table 1**
Chemical composition of native oat starch.

| Sample | Moisture (%) | Ash (%) | Protein (%) | Fat (%) | Amylose (%) |
|---|---|---|---|---|---|
| Starch | 8.23 ± 0.07 | 0.33 ± 0.01 | 0.28 ± 0.01 | 0.50 ± 0.07 | 28.7 ± 0.10 |

**Table 2**
Effects of acetic anhydride level on acetyl (%) and degree of substitution (DS) of oat starch.

| Acetic anhydride | Acetyl (%) | DS |
|---|---|---|
| Native starch | 0.00 | 0.00 |
| 6 (%) | $1.54^{a}$ | $0.05^{a}$ |
| 8 (%) | $2.92^{b}$ | $0.11^{b}$ |

Values with similar superscripts in a column differ significantly ($p < 0.05$).

**Table 3**
Effects of cross-linking and acetylation on swelling factor of oat starch.

| Starch | Swelling factor (%) |
|---|---|
| Native | $19.63^{c} \pm 0.15$ |
| Cross-linked (0.05%) | $13.23^{d} \pm 0.23$ |
| Cross-linked (0.1%) | $11.64^{c} \pm 0.28$ |
| Acetylated (6%) | $24.34^{b} \pm 0.34$ |
| Acetylated (8%) | $27.53^{a} \pm 0.10$ |

Values with similar superscripts in a column do not differ significantly ($p < 0.05$).

**Table 4**
Thermal characteristics of native, cross-linked and acetylated oat starch.

| Starch | $T_0$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $T_c - T_0$ (°C) | $\Delta H$ (J/g) |
|---|---|---|---|---|---|
| Native | 56.5 | 62.7 | 68.4 | 12.9 | 7.1 |
| Cross-linked (0.05%) | 55.8 | 62.6 | 68.6 | 12.8 | 7.4 |
| Cross-linked (0.1%) | 56.4 | 63.1 | 72.9 | 16.5 | 7.9 |
| Acetylated (6%) | 52.7 | 59.0 | 64.7 | 12 | 5.4 |
| Acetylated (8%) | 46.0 | 53.7 | 60.3 | 13.7 | 4.9 |

Compared with other cereal grains, oat starch has the greatest amount of lipids. The lipid content of oat starch in this study is lower than those in the results of Sowa and White (1992), because of different methods of lipid extraction and varietal differences.

### 3.2. Amylose content

Total amylose content of oat starch has been reported in the range of 27.5–29.5% (Tester & Karkalas, 1996) although Hoover and Vasanthan (1992) reported a lower value (19.4–22.7%) for amylose content of some Canadian oat starch. The corresponding value for oat starch examined in this study was 28.5–28.7% (Table 1) and these major discrepancies might be due to varietal differences. Both amylose and amylopectin participate in retrogradation, although amylose retrogradation is known to be a rapid process taking only a few hours, mostly due to its linear structure, that facilitates faster reassociation, while amylopectin retrogradation develops over a period of several days (Lawal, 2004).

### 3.3. Acetyl (%) and degree of substitution

The effect of addition of acetic anhydride at different levels (6% and 8%), on acetyl (%) and degree of substitution (DS) of oat starch is shown in Table 2. In acetylated oat starch, acetyl (%) and DS were lower than values reported earlier for potato and corn under similar conditions (Singh et al., 2004). This might be attributed to the difference of starch source, starch granular size and fragility and reaction conditions (Sodhi & Singh, 2005), such as rate of acetic anhydride addition, homogeneity in stirring and reaction time (González & Perez, 2002). Furthermore, González and Perez (2002) explained that the low degree of substitution in rice starch might be attributed to lack of granular surface pore or enough large inner channels which facilitate physical access of acetic anhydride to the interior of the granule. It was reported that acetyl content in acetylated starch with large granules was slightly higher than that of small granules (Van Hung & Morita, 2005). The oat starch granules were smaller and more rigid and packed more than were in potato and corn starch; therefore less substitution might occur.

### 3.4. Swelling factor

Cross-linking with $POCl_3$ led to reduction in swelling factor of oat starch (Table 3). Cross-linking strengthens the bonding between the starch chains, causing an increase in resistance of the granules to swelling with increasing degree of cross-linking (Singh et al., 2007). Cross-linking reinforces the structure of starch granules and limits water absorption by restricting the mobility of starch chains in the amorphous region (Gunaratne & Corke, 2007). Higher concentrations of fast-acting cross-linking reagents, such as $POCl_3$, result in greater reduction in swelling potential as compared with slower acting agents, such as EPI (Singh et al., 2007).

**Table 5**
Synaeresis (%) of native, cross-linked and acetylated oat starch.

| Treatment | Storage (days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 7 |
| Native | $9.66^{c} \pm 0.33$ | $11.54^{c} \pm 0.46$ | $13.14^{c} \pm 0.63$ | $14.22^{c} \pm 1.27$ | $14.70^{c} \pm 1.5$ |
| Cross-linked (0.05%) | $44.81^{b} \pm 0.82$ | $48.05^{b} \pm 1.77$ | $48.52^{b} \pm 1.77$ | $48.90^{b} \pm 1.76$ | $1.74 \pm 48.97^{b}$ |
| Cross-linked (0.1%) | $58.03^{a} \pm 0.25$ | $59.92^{a} \pm 0.20$ | $60.06^{a} \pm 0.22$ | $60.53^{a} \pm 0.05$ | $60.53^{a} \pm 0.05$ |
| Acetylated (6%) | $0.96^{d} \pm 0.06$ | $1.39^{d} \pm 0.09$ | $1.47^{d} \pm 0.09$ | $1.61^{d} \pm 0.10$ | $0.13 \pm 2.71^{d}$ |
| Acetylated (8%) | $0.04^{e} \pm 0.00$ | $0.04^{d} \pm 0.00$ | $0.09^{d} \pm 0.00$ | $0.29^{d} \pm 0.12$ | $0.30^{d} \pm 0.13$ |

Values with similar superscripts in a column do not differ significantly ($p < 0.05$).

Increase in level of added $POCl_3$ led to a fall in the swelling factor of modified starch that was due to the formation of a hard outer crust that restricted granule swelling (Kaur et al., 2006; Singh et al., 2007). Therefore, the paste made from a starch with an appropriate level of cross-linking is more viscose than that prepared from native starch. It is also stable to breakdown with extended cooking time, higher acid concentration and severe agitation (Kaur et al., 2006), making it suitable for use in retorted and high acidity foods. In contrast, swelling factor of acetylated starch was higher than that of native starch. The introduction of acetyl groups in rice starch could facilitate the access of water to amorphous areas, due to an intragranular structural disorganisation caused by steric effects and disruption of hydrogen bonds in the starch granules (González & Perez, 2002). It is feasible that, as the temperature of medium increased, starch molecules became more thermodynamically activated, and the resulting increase in granular mobility enhanced penetration of water (Lawal, 2004). Probably, the high swelling factor could increase water absorption of paste without explosion of granules that might lead to leaching out of amylose and retrogradation.

### 3.5. Thermal properties

Thermal properties of native and modified starch samples, e.g., onset temperature ($T_0$), peak temperature ($T_p$), concluding temperature ($T_c$) and enthalpy of gelatinization ($\Delta H$), which represent the amount of thermal energy involved in the gelatinization process, are presented in Table 4. This revealed that most of the corresponding temperatures of oat starch were not considerably changed with two concentration of $POCl_3$. Theoretically, reduction of swelling, resulting from cross-linking, should delay gelatinization; however, cross-linking had very little effect on gelatinization parameters (Gunaratne & Corke, 2007).

The cross-linking with $POCl_3$ did not change the onset and peak temperatures significantly, whereas the conclusion temperature and enthalpy increased with increasing degree of cross-linking; that had been reported in wheat starch, too (Van Hung & Morita, 2005). Cross-linking also altered the thermal transition characteristics of starch, although the effects depended on the concentration and type of cross-linking agent, reaction condition and the botanical source of the starch.

Acetylation reduced these parameters by weakening of the starch. Reduction in gelatinization temperatures after derivatisation led to the structural reorganization and consequent weakening of intragranular and intergranular binding forces of the starch molecules. Enthalpy of gelatinization gives an overall measure of crystallinity and is an indicator of the loss of molecular order (Singh et al., 2007). The decrease in $\Delta H_g$ suggests that the acetyl group disrupts double helices within amorphous regions of the granule. Also, the introduction of bulky groups into the backbone of the biopolymer enhances structural flexibility, and this also contributes to reduction of gelatinization temperature of the modified starch. Decrease in thermal parameters is consistent with fewer crystals being present after modification, with a co-operative melting process enhanced by added swelling.

### 3.6. Synaeresis

The retrogradation of native and cross-linked starch pastes, increased significantly during refrigerated storage (Table 5). Synaeresis in the stored gels is due to the increased molecular association between the starch chains at reduced temperature, excluding water from the gel structure. Retrogradation properties of starch are indirectly influenced by structural arrangements of starch chains within the amorphous and crystalline regions of the ungelatinized granule, which in turn, influence the extent of granule breakdown during gelatinization and the interactions that occur between starch chains during gel storage (Kaur et al., 2006). Furthermore, the increase in synaeresis during storage has been attributed to the interaction between leached out amylose and amylopectin chains.

Amylose aggregation and crystallization were completed within the first few hours of storage while amylopectin aggregation and crystallization occurred during later stages (Sodhi & Singh, 2005). Cross-linking resulted in an ordered structure of the starch pastes,

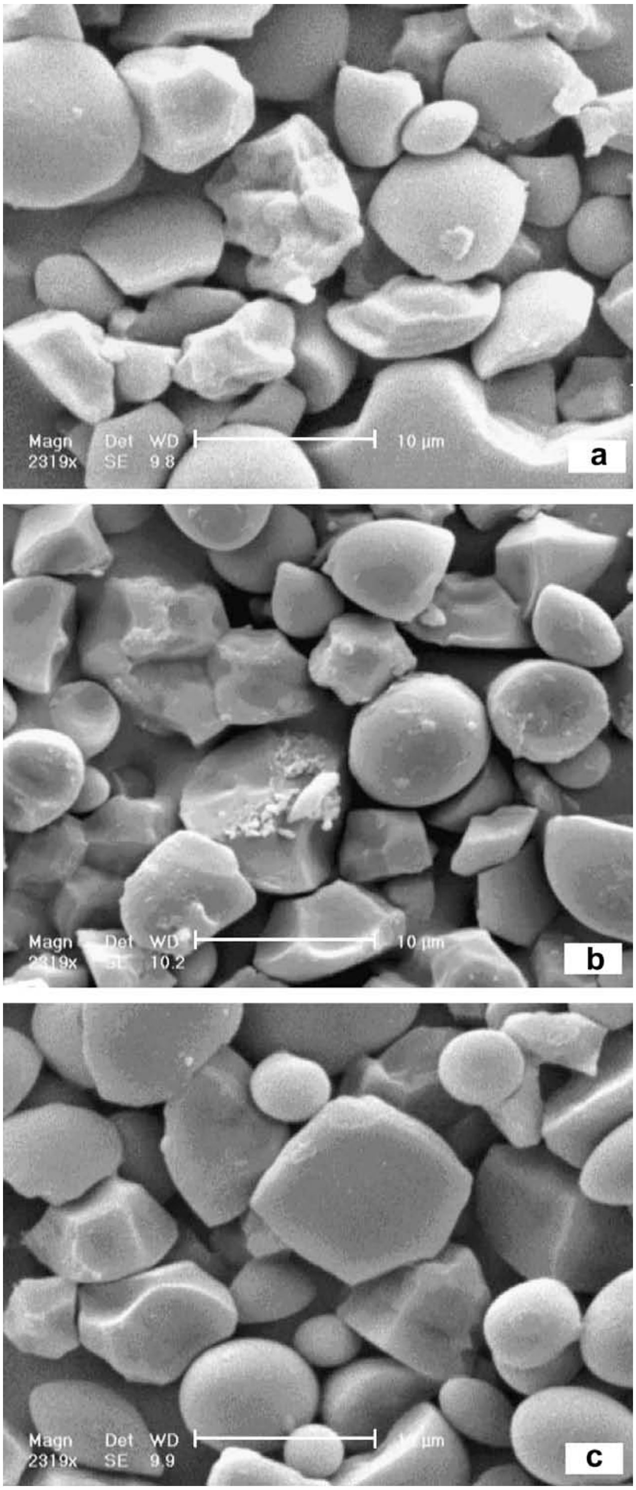

**Fig. 1.** Scanning electron micrographs of native (a), cross-linked (b) and acetylated (c) oat starch.

thus resulting in higher degree of retrogradation. As the level of cross-linking increased, the paste became more unstable in low temperature conditions. In contrast, acetylation decreased synaeresis due to the presence of acetyl groups on the starch molecules, that are able to increase water retention capacity of refrigerated stored gels (Sodhi & Singh, 2005).

### *3.7. Morphological characteristics*

The appearance of starch granules was not affected by exo-erosion due to chemical modification processes (either cross-linking or acetylation) that may be due to their small granule size (Fig. 1). Van Hung and Morita (2005), in a study on the effect of granule size on physicochemical properties of cross-linked and acetylated wheat starch, showed that surfaces of the large granules suffered greater damage after modification than did those of the small granules. As for the large granule, cross-linking affected the surface of the granules only slightly, whereas acetylation damaged the whole surface of granules. However, the small effect of acetylation on surfaces of oat starch granules might be due to the small granular size.

## 4. Conclusion

The physicochemical properties of cross-linked and acetylated oat starch were investigated. Cross-linking decreased the swelling factor whereas it increased synaeresis. Gelatinization temperature of starch was not significantly affected after cross-linking. Acetylation increased the swelling factor but reduced gelatinization temperature and synaeresis of oat starch. Appearances of oat starch granules were not affected after cross-linking and acetylation. Therefore, level of cross-linking that allows optimal performance is very important in a particular system. Considering these characteristics of acetylated starch, it could potentially be used in different food products, including baked goods, canned pie fillings, sauces, retorted soups, frozen foods, baby foods, snacks and salad dressings.

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