

Assessment of different methods for determining the capacity of water absorption of ingredients and additives used in the meat industry

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Abstract

Water absorption capacity (WAC) basically consists in the method for quantify the water retained by the pelleted material in the centrifuge tube after adding water or an aqueous solution to a material. This property is economically important because it affects the yield and quality of meat products (cooked hams, sausages and mortadellas). The aim of this study was to evaluate three methods for determining WAC and to compare the values obtained for thirteen different ingredients (proteins and polysaccharides) used in the meat industry. There was a significant difference ($p < 0.05$) in the WAC values (%) obtained for the three methods. Only the cassava starch showed similar % WAC results, regardless of the evaluation method used. The highest percentages of WAC were obtained for a sample of modified starch with up to 666.62%, and also 648.22% for guar gum and 573.90% for soy protein isolate. Method 1 (addition of water) showed results that were most consistent with the literature data and higher WAC values for seven of the thirteen samples tested, thus this was the best method.

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Keywords

Guar gum

Modified starch

Cassava starch

Soy protein

Carrageenan

Introduction

Water absorption capacity (WAC) or water holding capacity (WHC) consists of adding water or an aqueous solution to material, followed by centrifugation and quantification of the water retained by the pelleted material in the centrifuge tube (Damoradan *et al.*, 2010).

WAC is economically important for the meat-processing industry because the loss of moisture adversely affects the yield and quality of the product (Ordóñez *et al.*, 2005). According to Wang *et al.* (2006) high values of water absorption capacity are important to help maintain the moisture content of products.

The use of ingredients with high WAC assists in improving the quality of meat products and the maintenance of appropriate characteristics throughout shelf-life or even when the product is subjected to adverse conditions such as high temperatures and freezing (for example as ready-made dishes or foods that go from freezer to oven). Proteins and

polysaccharides are among the ingredients and additives commonly used by the industry. However, there is a gap in the literature regarding studies assessing WAC ingredients and additives, as well as the most appropriate methodology for this assessment. There is also a need to generate information for industry in order to assist in the choice of ingredients and additives in the development of new formulations of meat products.

Different methods for evaluating WAC are described in the literature (Barbut, 1996; Tsai *et al.*, 1998; Hedenus *et al.*, 2000; Wang *et al.*, 2006). Wang *et al.* (2006) reported the methodology developed by Sosulski (1962), which determines the absorption of water in flours by centrifugation and drying in an oven. Tsai *et al.* (1998) suggest only centrifuging, without the need for drying. Hedenus *et al.* (2000) suggest two methods to assess the absorption of water; one using isotherms and the other by evaluating the volume before and after swelling. The aim of the present study was to evaluate and compare the WAC of various ingredients and additives used in the meat

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industry using three different methods to determine which ingredient or group had a greater WAC and whether there would be differences between them.

Materials and Method

The experiments were performed at the Laboratory of Physical Chemistry, Department of Food Science and Technology (DTCA) of the Federal University of Santa Maria (UFSM), Santa Maria (RS), Brazil. The ingredients and additives were divided into two groups (proteins and polysaccharides) and the following were tested: (SIP) soy protein isolate (ADM, São Paulo, SP); (TSP1) textured soy protein (Doremus, Guarulhos, SP); (TSP2) textured soy protein (Marsul, Montenegro, RS); (CSP) concentrated soy protein (Marsul, Montenegro, RS); (GG) guar gum (Nutract, Chapecó, SC); (CAR) kappa-carrageenan (Doremus, Guarulhos, SP); (U8) Ultratex 8 modified starch (National Starch, Jaguaré SP); (MS1) Novation 2300/EK 8925 modified starch (National Starch, Jaguaré SP); (EMS) Eliane 100 modified starch (National Starch, Jaguaré SP); (TMS) Thermetex modified starch (National Starch, Jaguaré SP); (MS2) Novation 1900 modified starch (National Starch, Jaguaré SP); (CS) cassava starch (Lar, Medianeira, PR) and (U3) Ultratex 3 modified starch (National Starch, Jaguaré SP).

The pH of all the ingredients and additives was evaluated by electrometric determination according to the official method (AOAC, 1990; IAL, 2005) in water and in saline solution (0.6M). Different solvents were tested to evaluate the differences and effects on the WAC of the ingredients and additives.

For the evaluation of WAC, three methods were used to assess which method was more appropriate and/or which would present most consistent results and also which would be most suitable to be used in the meat industry meats where maximum WAC is required. Method 1 consisted of weighing 5 g of sample into each centrifuge tube (Falcon type plastic tubes with capacity of 50 mL) and adding 32 mL of distilled water followed by manual shaking for 1 minute. Immediately afterwards, the tubes were placed at rest for 10 minutes and then centrifuged for 25 minutes at 2,900 g. The supernatant was discarded and the tubes were dried in an oven with circulating air (50°C for 20 minutes with downward inclination of 15° to 20°). After drying, the tubes were weighed again and the WAC was calculated for each sample as a percentage, taking into consideration the difference in weight, according to Sosulski's modified methodology (1962) cited by Wang *et al.* (2006).

Method 2 consisted of weighing 5 g of sample

Table 1. pH results for the evaluated ingredients and additives in water or 0.6M saline solution)

| Groups/Classes | Samples | pH | pH |
|-----------------|---------|---------------------------|--------------------------|
| Proteins | SIP | 6.93 ^{Ab} ±0.02 | 6.79 ^{Ad} ±0.02 |
| | TSP1 | 5.92 ^{Ac} ±0.02 | 6.24 ^{Bc} ±0.02 |
| | TSP2 | 6.93 ^{Ab} ±0.01 | 7.49 ^{Bb} ±0.07 |
| | CSP | 6.42 ^{Aef} ±0.19 | 7.03 ^{Af} ±0.20 |
| Polysaccharides | TMS | 6.75 ^{Bg} ±0.04 | 6.19 ^{Ad} ±0.02 |
| | U3 | 5.18 ^{Ab} ±0.03 | 5.22 ^{Bb} ±0.01 |
| | U8 | 5.24 ^{Ab} ±0.01 | 5.30 ^{Ab} ±0.01 |
| | EMS | 6.69 ^{Afg} ±0.30 | 6.44 ^{Ad} ±0.28 |
| | MS1 | 5.55 ^{Abc} ±0.02 | 4.78 ^{Aa} ±0.02 |
| | MS2 | 4.27 ^{Aa} ±0.01 | 4.87 ^{Bb} ±0.01 |
| | CAR | 8.73 ^{Ai} ±0.05 | 9.63 ^{Bb} ±0.05 |
| | GG | 6.14 ^{Ad} ±0.01 | 5.88 ^{Ac} ±0.01 |
| | CS | 7.59 ^{Bh} ±0.04 | 6.01 ^{Ad} ±0.04 |

Means with different lowercase letters vertically differ significantly ($p < 0.05$). Means with capital letters horizontally differ significantly ($p < 0.05$). The abbreviations correspond to: (SIP) isolated soy protein, (TSP1) textured soy protein 1, (TSP2) textured soy protein 2, (GG) guar gum, (CSP) concentrated soy protein, (CAR) kappa carrageenan, (U8) modified starch, (MS1) Novation 2300/EK 8925 modified starch (MS2), Novation 1900 modified starch (MS2), (EMS) Eliane 100 modified starch, (TMS) Thermetex modified starch, (CS) cassava starch and (U3) Ultratex 3 modified starch.

and adding 8 mL of 0.6M NaCl solution. This was homogenized and subsequently left to stand in an ice bath for 30 minutes. The material was subsequently centrifuged at 2,900 g for 5 minutes at room temperature. After centrifugation the supernatant was discarded and the tube was reweighed to obtain the weight of saline solution that was retained. The results were expressed as a percentage of the retained solution according to the methodology described by Barbut (1996).

Method 3 consisted of weighing 5 g of sample and adding 32 mL 0.6M NaCl solution. This was homogenized and subsequently left to stand in an ice bath for 30 minutes. The material was subsequently centrifuged at 2,900 g for 5 minutes at room temperature. After centrifugation, the supernatant was discarded and the tube was reweighed to obtain the weight of saline solution that was retained, according to the methodology described by Barbut (1996) with modifications.

The experimental design was completely randomized and there were three replicates for each test for each ingredient or additive. The results were submitted to analysis of variance - ANOVA and Tukey's test with a significance level of 95% ($p < 0.05$) using the Statistica® 8.0 (STATSOFT, INC) software.

Results and Discussion

Table 1 shows the results obtained for the pH of the ingredients and additives tested in water (traditional method) and saline solution (0.6 M). There was

a significant difference ($p < 0.05$) for pH between samples and also between methods. The protein SIP had the highest pH value (6.93) and the sample MS2 had the lowest (4.27) when evaluated in water. In terms of the saline solution, CAR had the highest pH value (9.63) and the MS1 starch had the lowest (4.78). In general, proteins showed higher pH (6.72) when compared with polysaccharides (5.87), regardless of the solvent used. TSP1 and TSP2, U3, CS and MS2 starches, and CAR showed the highest pH and only the TMS starch showed reduced pH with the use of saline as a solvent. For SIO, CSP, U8, EMS, MS1 and GG there was no significant change ($p > 0.05$) in pH when saline solution was used. Comparing the native starch (CS) with the modified starches (TMS, U3, U8, EMS, MS2 and MS1) the modified starches mostly showed lower pH values, which was probably due to the modification processes.

The addition of salt can lead to proximity of the IP (isoelectric point) of the proteins and it alters the solubility of the proteins because it modifies the distribution of hydrophobic and hydrophilic amino acids on the surface of the protein. The change in pH alters the distribution of the cationic, anionic and non-ionic polar sites in the protein molecules, which affects the water-protein and protein-protein interactions (Araújo, 2008).

The behavior of protein solutions is markedly affected by the presence of ions of low molar mass, especially the anions and cations in salts. At certain concentrations sodium chloride enhances the solubility of the proteins and a process called 'salting-in' occurs, in which the charged groups on the surface attract and bind anions and cations more strongly than with the water. However, these ions still bring with them an ordered group of their own water molecules of solvation, which maintain the protein molecules in solution. Higher concentrations of electrolytes promote the precipitation of proteins in solution, a phenomenon known as 'salting-out'. There is competition between ions in the salt with the protein for the water necessary to keep the protein in solution, so that if the salt concentration is raised the proteins will precipitate (Coulter, 2004).

The addition of salts to polysaccharide solutions can promote changes by increasing or decreasing solubility. The variation in the degree of the substituents of a polysaccharide changes its rheological properties in solution, whose behavior can be attributed to the interaction between charged residues and ions from the dissociation of salt, when present in solution. This behavior may indicate that a more extended chain promotes solubility and leads to a lower viscosity because the charge density

Table 2. Water Absorption Capacity (WAC) in % for the different evaluated ingredients or additives

| Groups/Classes | Samples** | WAC (%) | | |
|-----------------|-----------|---------------------------|---------------------------|---------------------------|
| | | Method 1 | Method 2 | Method 3 |
| Proteins | SIP | 573.90 ^C ±0.57 | 160.46 ^A ±0.30 | 341.37 ^B ±0.32 |
| | TSP1 | 205.60 ^C ±0.14 | 160.99 ^A ±0.79 | 194.36 ^B ±0.44 |
| | TSP2 | 351.50 ^C ±0.71 | 160.90 ^A ±0.64 | 284.75 ^B ±0.29 |
| | CSP | 313.50 ^C ±0.99 | 159.10 ^A ±0.40 | 296.33 ^B ±0.15 |
| | TMS | 74.98 ^A ±0.17 | 91.55 ^B ±0.16 | 93.84 ^C ±0.76 |
| | U3 | 468.55 ^B ±0.50 | 162.27 ^A ±0.57 | 666.62 ^C ±0.61 |
| | U8 | 547.55 ^C ±0.71 | 161.05 ^A ±0.71 | 530.20 ^B ±0.87 |
| | EMS | 74.79 ^A ±0.22 | 74.15 ^{Ab} ±0.64 | 81.87 ^B ±0.79 |
| Polysaccharides | MS1 | 121.40 ^C ±0.57 | 77.08 ^A ±0.47 | 108.98 ^B ±0.48 |
| | MS2 | 81.70 ^{Ab} ±0.40 | 88.96 ^B ±0.97 | 102.97 ^C ±0.38 |
| | CAR | 231.40 ^C ±0.85 | 161.40 ^A ±0.26 | 227.24 ^B ±0.36 |
| | GG | 621.55 ^B ±0.64 | 160.89 ^A ±0.37 | 648.22 ^C ±0.36 |
| | CS | 72.47 ^A ±0.06 | 68.10 ^A ±0.82 | 72.54 ^A ±0.55 |

Means with different lowercase letters vertically differ significantly ($p < 0.05$). Means with capital letters horizontally differ significantly ($p < 0.05$). The abbreviations correspond to: (SIP) isolated soy protein, (TSP1) textured soy protein 1, (TSP2) textured soy protein 2, (GG) guar gum, (CSP) concentrated soy protein, (CAR) kappa carrageenan, (U8) modified starch, (MS1) Novation 2300/EK 8925 modified starch (MS2), Novation 1900 modified starch (MS2), (EMS) Eliane 100 modified starch, (TMS) Thermetex modified starch, (CS) cassava starch and (U3) Ultratex 3 modified starch.

(number of charges per unit of length) modifies the macromolecular properties in solution (Aranda-Selverio *et al.*, 2010). Ramaswamy *et al.* (2013) reported that chains of arabinans with a linear form showed greater water retaining capacity than branched chains that can be explained by the higher mobility of the molecules in solution, which allows greater interaction with water.

Table 2 shows the results obtained for the WAC (%) of the tested ingredients and additives using three different methods. A significant difference ($p < 0.05$) was observed in the WAC between the tested ingredients and additives and between the different tested methods, except for CS.

The proteins had an average WAC (%) of 361.13%, 160.36% and 279.20% for Methods 1, 2 and 3, respectively. The polysaccharides in Method 1 had an average WAC of 254.93%, 116.17% for Method 2 and 281.39% for Method 3. The highest WAC % were obtained for SIP, with up to 573.90% using Method 1, and U3, with 162.27% for Method 2 and 666.62% for Method 3. The lowest WAC % were found for CS using Methods 1, 2 and 3, at 72.47%, 68.10% and 72.54%, respectively.

For Method 1, it was found that GG showed the highest water absorption, reaching 621.55% (about 6 times its weight in water.). Because this method indicates the addition of 32 mL of water in 5 g of sample, maximum hydration is 640% and therefore GG reached values close to the limit. Using this same

relationship for Methods 2 and 3, the maximum WAC would be 160% and 640%, respectively. For Method 2, the maximum hydration value was obtained for various tested ingredients and additives (SIP, TSP1, TSP2, U3, U8, CAR and GG) with no statistically significant difference ($p > 0.05$).

The high value of WAC presented by GG was close to that found by Prestes *et al.* (2012), which was 615.12%, and Prestes (2008), 548.10%. WAC presents a linear structure and GG has a linear structure with branches of simple units and therefore exhibits behavior similar to a linear polymer. Both have high solubility in water (Damoradan *et al.*, 2010). As for carrageenan, Prestes (2008) obtained values of absorption of up to 336.8% and Prestes *et al.* (2012) obtained about 241.30%. These differences may be explained by the physico-chemical characteristics and type of carrageenan that was evaluated (kappa, iota or lambda) and also by the presence of impurities.

Analyzing the soy proteins, the highest values of WAC (%) were found for SIP (Methods 1 and 3) due to its larger surface area caused by the finer particles (dust) in relation to the other types of protein (flakes or granules) and also by the increased solubility of this protein resulting from the purification process and requirement to reach industrial level. The TSP1 and TSP2 proteins differed in WAC (Methods 1 and 3) and in pH (in water and in saline solution). These differences may be explained by differences in particle size, protein content, and possible variations in obtaining these proteins. Prestes *et al.* (2012) found WAC values ranging from 223.95% to 309.38% for TSP (using the same Method 1), values close to those found in this present study. Damoradan *et al.* (2010) refer to water absorption of 33% for soy protein.

The amino acid residues located on the surface of the proteins are responsible for the acid or basic behavior and the solubility, varying with pH and temperature, ionic strength and dielectric constant. Each amino acid has a side chain characteristic that influences the physico-chemical properties of the proteins. According to Damoradan *et al.* (2010), the higher the amount of charged residues the greater the ability to bind to water.

The difference between the methods employed is in the addition of saline in Methods 2 and 3, in smaller and larger proportions respectively, with the aim of submitting the ingredients or additives to conditions closer to that used in the meat industry, which mostly consists of high salt concentrations.

Comparing the WAC obtained for the proteins using Method 3 in relation to Method 2, it was observed that the addition of high concentration saline solution (0.6 M) caused a reduction in WAC,

especially for SIP (approximately 40.00 % less than the maximum obtained). This was probably because there was a higher protein-protein interaction, unlike that which may have occurred in Method 2.

On the other hand, it was observed that there was no significant difference ($p > 0.05$) between the proteins (SIP, TSP1 and TSP2) using Method 2, which can be explained by the low amount of water added (8 mL) compared to the mass of sample (5 g), which led to maximum possible hydration (160%). In this case, the proteins retained greater interaction with the water.

Araújo (2008) states that when the pH is close to the isoelectric point of the protein, and/or there is the addition of salt or solvents, a change occurs in the solubility of the protein. At the pH closest to the isoelectric point, the forces of attraction are predominant and the capacity for water absorption decreases. Some proteins precipitate in the presence of salt and their solubility diminishes. This occurs because of the competition between the protein and the salt ions for the water molecules, which consequently leads to the removal of the water by hydration of the protein, resulting in a greater protein-protein interaction that becomes stronger than the protein-water interaction, and thus the aggregation of the protein molecules occurs, followed by precipitation. However, for most proteins, low salt concentration ($< 0.2M$) increases solubility (in certain pH and temperatures) (Damoradan *et al.*, 2010). With the addition of salt, the electrostatic repulsion increases and the loosening of the protein molecules occurs.

The majority of polysaccharides contain glycosyl units that have three hydroxyl groups in their structure. Each of these groups has the possibility to form hydrogen bonds with one or more molecules of water. In aqueous systems, particles of polysaccharide molecules can capture water, swell, and generally undergo partial or complete dissolution. The water binds to the molecules of the polysaccharides by hydrogen bonds. In general, the polysaccharides become more soluble depending on the degree of irregularity of the molecular chains, i.e. the greater the difficulty for the molecules to bind, the higher the hydration (Damoradan *et al.*, 2010).

For CS, the low WAC can be justified due to the fact that starch granules are insoluble and only hydrate very slightly in cold water (Damoradan *et al.*, 2010). The higher absorptions for the modified starches (U8, U3 and MS1) were highlighted when compared with the native starch (CS). Wang *et al.* (2006) found an absorption rate of 60% for wheat flour, a value close to that found for CS using Method 2. Modified starches undergo physical or chemical

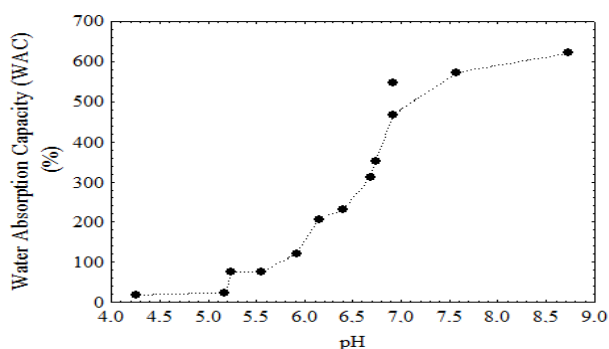


Figure 1. Relationship between pH values (in water) and WAC (%) using Method 1

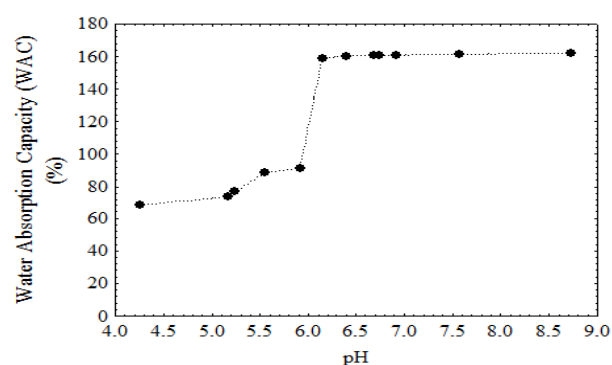


Figure 2. Relationship between pH (in 0.6 M saline) and WAC (%) using Method 2

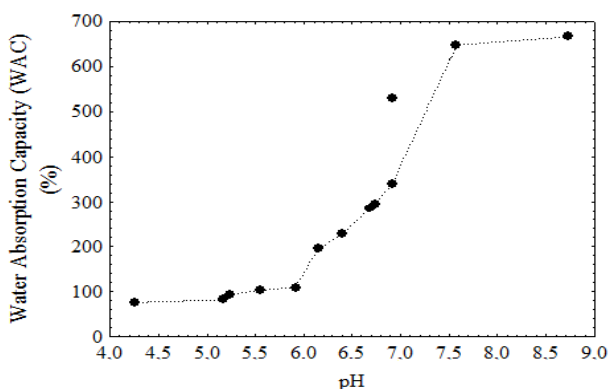


Figure 3. Relationship between pH (in 0.6 M saline) and WAC (%) using Method 3

changes (Baruffaldi and Oliveira, 1998). Chemical modifications result in products that are crosslinked, stabilized, oxidized and depolymerized, and result in products that are dispersible in cold water. Any starch (corn, waxy maize, potato, cassava, wheat, rice, etc.) may be modified, but in general, waxy maize starch, cassava and maize are most commonly used (Silva *et al.*, 2006; Damoradan *et al.*, 2010; Demiate and Kotovicz, 2011).

Changing the starch allows the possibility of mixtures that can be used in large commercial applications, such as maltodextrins, maltose and glucose syrup concentrates. These products are widely used in the food industry (Franco *et al.*, 2001). Prestes (2008) studied modified corn starch and the

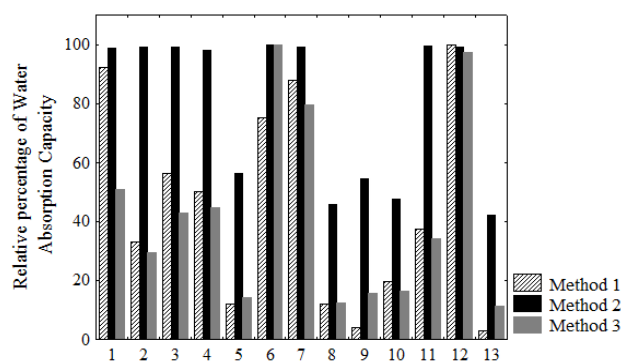


Figure 4. Comparison of methods in relation to the maximum WCA % for the thirteen ingredients and additives tested. 1 corresponds to (SIP) isolated soy protein; 2 to (TSP1) textured soy protein 1; 3 to (TSP2) textured soy protein 2; 4 to (CSP) concentrated soy protein; 5 to (TMS) Thermetex modified starch; 6 to (U3) Ultratex 3 modified starch; 7 to (U8) modified starch; 8 to (EMS) Eliane 100 modified starch; 9 to (MS1) Novation 2300/EK 8925 modified starch; 10 to (MS2) Novation 1900 modified starch; 11 to (CAR) kappa carrageenan; 12 to (GG) guar gum, and 13 to (CS) cassava starch.

WAC values reached 92.01%. Modified starches are used by the industry in ready meals in order to reduce syneresis and the release of fluids during transport and processing.

All the methods used in the present study showed a tendency to increase the WAC % depending on the pH of the ingredients or additives for all the tested methods (Figures 1, 2 and 3), however Method 1 presented the results that were most consistent with the data found in the literature (Wang *et al.*, 2006; Damoradan *et al.*, 2010; Prestes *et al.*, 2012) and higher WAC values for seven of the thirteen ingredients studied. The highest WAC % were obtained for the ingredients and additives with higher pH levels, regardless of the method tested. These results can be explained by the proximity of the isoelectric point in the case of the proteins, and regarding the polysaccharides, it may be due to some straight-chain polysaccharides exhibiting only one type of ionic charge (often negative) which leads them to assume an extended configuration due to the repulsion of charges of the same sign, which can reduce the absorption of water (Damoradan *et al.*, 2010).

Based on the results obtained in the present study, the higher WAC values of the proteins, starches and modified starches are noteworthy, and demonstrate the potential use of these ingredients or additives in industrial products requiring high water retention. The large variations of WAC within the same group, and even between the same ingredients/additives are directly related to the process of obtaining these data and these variations may influence the final product,

mainly in technological aspects (syneresis, brittle texture, etc.).

For both classes (proteins and polysaccharides), Method 2 resulted in a reduction of WAC for most of the ingredients or additives due to the lower availability of water for hydration. When Methods 1 and 3 were compared, the results only showed a relevant increase in WAC % for the U3 modified starch and a reduced absorption for all the proteins for CAR gum and MS1 modified starch. The increase in WAC for U3 can be explained by the interaction between charged residues and ions from the dissociation of salt, which, when present in solution, provide a more extended chain that favors solubility, as suggested by Aranda-Selverio *et al.* (2010).

Figure 4 shows that only GG reached maximum WAC for all the methods. For Method 2, due to the lower amount of water for hydration, most of the ingredients and additives reached maximum WAC (of the method). As for Method 1, SIP, and U3 and U8 modified starches reached the maximum hydration. The TMS, EMS, MS2, MS1 modified starches and the FM native starch showed absorption less than 60% for all methods.

Conclusion

There is a lack of studies evaluating the differences between the methods and the differences of WAC for various ingredients. These results can help in the development of new formulations of meat products at the industrial level, reducing testing time and facilitating the choice of the best or most appropriate ingredient/additive according to the application and/or method used. The highest WAC values were found for the modified starch (U3) (666.62%), guar gum (GG) (648.22%) and soy protein isolate (SIP) (573.90%). The highest WAC % were obtained with ingredients or additives with higher pH, independent of the tested methods. Method 1 (addition of water, centrifugation and drying) showed the results that were most consistent with the literature data and higher WAC values for seven of the thirteen tested samples and therefore would be the most recommended method.

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