International Food Research Journal 23(2): 653-659 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Evaluation of water absorption capacity of ingredients and additives used in the meat industry submitted to different saline concentrations and ultrasound

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Article history

Received: 19 September 2014
Received in revised form: 21 August 2015
Accepted: 26 August 2015

Keywords

Guar gum
Modified starch
Cassava starch
Soy protein
Carrageenan

Abstract

This study evaluated the water absorption capacity (WAC) of thirteen samples of ingredients and additives, which were divided into two groups (proteins and polysaccharides) by the addition of different concentrations of saline solutions, and exposure to ultrasound. Concentrations of 2, 4, 6 and 8% (w/v) sodium chloride were tested in order to simulate industrial usage in brine. For the treatments with ultrasound, a 2^2 factorial design (0 or 2% salt and 15 or 30 min of exposure to ultrasound bath) was used. The method for evaluating WAC (%) consisted of the addition of the solution, centrifugation, drying and weighing. The highest level of WAC was found for guar gum (639.96% to 8% NaCl), and the lowest was for cassava starch (67.08% to 2% NaCl). Both the groups showed an increase in WAC, with an increase in the salt concentration in the added solution. The treatments with ultrasound showed an increase in WAC for most of the polysaccharides that were tested. Comparing Treatment 3 (2% NaCl, 15 min) and Treatment 4 (2% NaCl, 30 min) with Treatment 1 (0% NaCl, 15 min) and Treatment 2 (0% NaCl, 30 min) it was observed that the concomitant addition of salt with the use of ultrasound only increased the WAC of one of the thirteen samples that were evaluated. These results can help in the development of new formulations of meat products, and the tested conditions and the use of ultrasound proved to be an interesting alternative to increase WAC, mainly for the polysaccharides that were evaluated.

Introduction

Water absorption capacity (WAC) or water holding capacity (WHC) consists of adding water or an aqueous solution to a material by centrifugation and quantifying the water that is retained in the pelleted material in the centrifuge tube (Damoradan *et al.,* 2010). According to Wang *et al.* (2006), high WAC values are important in order to help maintain moisture content in products. Moisture loss adversely affects the yield and quality attributes of meat products, and therefore results in economic losses for industries (Ordóñez *et al*., 2005).

Different methods for the evaluation of WAC are described in the literature (Barbut, 1996; Tsai *et al*., 1998; Hedenus *et al*., 2000; Wang *et al*., 2006). However, there is a gap in relation to studies assessing the WAC of ingredients and additives when they are subjected to different salt concentrations or brine, which are the most common forms of usage of ingredients and additives in meat products. There are also few studies that have evaluated the effects of the use of ultrasound on the WAC of polysaccharides and proteins (Hu *et al.*, 2013).

The use of ultrasound in the food industry has attracted attention (Wu *et al*., 2011; Hu *et al*., 2013) because it can improve process efficiency and dissemination, reduce time, and contribute to cleaner production, as well as lower power consumption (Sivakumar and Rao, 2003; Sivakumar *et al*., 2010). Ultrasound can be applied to change the physical and chemical properties of food (Chemat and Khan, 2011, Pingret *et al*., 2013) in the processing stages, in the preservation and extraction processes, as well as

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in combination with other processes (Yu *et al*., 2013). In a study by Hu *et al*. (2013) the pre-treatment of soy protein isolate with high-intensity ultrasound (20 k Hz, 400 W) improved the WAC and the strength and firmness of the gels. The sonication times (0, 5, 20, and 40 min) had significant effects on these properties.

Ultrasound uses the energy of sound waves, which are generated at a frequency higher than the hearing ability of humans (> 16 k Hz) (Sayasooriya *et al*., 2004). These sound waves create a change in fluid pressure and generate cavitation. Cavitation bubbles are formed during sonication and undergo a violent collapse, which leads to extreme temperatures and pressures; this results in turbulence and shear in the cavitation zone. Treatment with ultrasound can also lead to the formation of highly reactive free radicals due to breakage of the water molecules, and these radicals can react with other molecules (Hu *et al*., 2013). Thus, the aim of this study was to evaluate and compare the WAC (%) of various ingredients and additives used in the meat industry, which were subjected to different saline concentrations and exposure to ultrasound.

Materials and Methods

The experiments were performed in the laboratories of the Department of Food Science and Technology (DTCA) of the Federal University of Santa Maria (UFSM) in Santa Maria (RS, Brazil). The ingredients and additives were separated into two groups (proteins and polysaccharides) and the following were tested: (SPI) soy protein isolate with a particle size of 0.149 mm (90% retained on 100 mesh sieve) (ADM, São Paulo, SP, Brazil); (TSP1) textured soy protein with a particle size of 0.250 mm (85% retained on 60 mesh sieve) (Doremus, Guarulhos, SP, Brazil); (TSP2) textured soy protein with a grain size between 0.149 mm (20% retained on 100 mesh sieve) and 0.355 mm (95% retained on 42 mesh sieve) (Marsul, Montenegro, RS, Brazil); (CSP) concentrated soy protein <0.149 mm (5% retained on 100 mesh sieve) (Marsul , Montenegro, RS, Brazil); (GG) guar gum (Nutract, Chapecó, SC, Brazil); (CAR) kappa-carrageenan (Doremus, Guarulhos, SP, Brazil); (ULTRATEX8), Ultratex 8 modified starch (National Starch, Jaguaré, SP, Brazil); (NOV1900) Novation 1900 modified starch (National Starch, Jaguaré, SP, Brazil); (MS) Eliane 100 modified starch (National Starch, Jaguaré, SP, Brazil); (THERM) Thermetex modified starch (National Starch, Jaguaré, SP, Brazil); (NOV2300) Novation 2300/EK 8925 modified starch (National Starch, Jaguaré, SP, Brazil); (CS) cassava starch (Lar, Medianeira, PR, Brazil); and (ULTRATEX3) Ultratex 3 modified starch (National Starch, Jaguaré, SP, Brazil).

Table 1. 2^2 factorial design used for the experiments to evaluate WAC (%) with treatment with ultrasound and saline solution (real and coded values)

Treatments	TRT1	TRT2	TRT3	TRT4
Time (min)	15	30	15	30
	(-1)	(-1)	(+1)	(+1)
Salt (NaCl) concentration (%)	0.0	0.0	2.0	2.0
	(-1)	(+1)	(-1)	(+1)

For the evaluation of WAC, the method consisted of weighing 1:56 g of sample for each centrifuge tube (Falcon plastic tubes type with a capacity of 15 mL) and 9.6 mL of saline solution was then added. For the preparation of the solutions, sodium chloride (≥ 99.5%) (Sigma-Aldrich, São Paulo, SP, Brazil) was used at concentrations of 2, 4, 6 and 8% (m/v) to conform to saline concentrations used industrially. After the addition of the solution, the tubes were manually shaken for about 1 minute. Soon after, the tubes were placed to rest for 10 minutes and then centrifuged for 25 min at 2,900 g (ITR centrifuge, Super II model, Instrumentos para Laboratórios LTDA, Esteio, RS, Brazil). The supernatant was discarded and the tubes were dried in an oven with air circulation at 50°C for 20 minutes with downward tilt of 15° to 20° (Marconi oven, MA033 model, Piracicaba, SP, Brazil). The purpose of drying after centrifugation was to remove the excess of brine that was not discarded as supernatant. After drying, the tubes were weighed again and the WAC (%) was calculated for each sample by taking into consideration the difference in weight, as in the modified methodology of Sosulski (1962) cited by Wang *et al*. (2006).

For the experiments with ultrasound, the samples in the centrifuge tubes, with solution already added, were subjected to different times (15 or 30 min), and salt concentrations (0 or 2%) to conform with a full 2^2 factorial design, which is presented in Table 1. Times of 15 or 30 minutes were used, based on the study by Jambrak *et al.* (2010). The samples were subjected to ultrasound (without heat) after the rest period, and the evaluation of WAC was subsequently performed according to the methodology described above. An ultrasonic bath was used, which is commonly used for

Table 2. Water absorption capacity (WAC) in % evaluated for the evaluated additives or ingredients (proteins and polysaccharides) in the different tested saline concentrations.

Groups/Concentrations*		2 %	4 %	6 %	8 %
Proteins	SPI	302.75^{gA}±1.50	317.87^{gA}±8.27	313.32^{gA}±4.89	316.91^{gA}±1.84
	TSP1	187.96^{eA}±2.68	220.67^{dB}±4.43	190.89^{eA}±2.31	209.97^{eB}±8.23
	TSP2	238.43^{fAB}±8.09	293.28^{fB}±2.75	194.06^{eA}±3.57	212.00^{eAB}±8.09
	CSP	315.66^{hB}±4.61	265.18^{eA}±9.06	254.82^{fA}±3.47	368.26^{hC}±3.23
Polysaccharides	THERM	87.05^{bA}±0.67	87.31^{aA}±0.99	89.90^{bB}±0.06	85.80^{bA}±0.14
	ULTRATEX8	332.84^{iD}±0.46	272.85^{eC}±0.96	264.02^{fB}±1.43	259.47^{fA}±0.32
	ULTRATEX3	512.16^{jB}±3.18	513.51^{hB}±5.13	194.51^{eA}±4.17	515.32^{iB}±4.91
	MS	86.05^{bC}±0.74	81.36^{aAB}±0.84	79.63^{abA}±0.42	83.46^{abB}±0.43
	NOV1900	112.15^{cB}±0.49	106.04^{bA}±1.60	125.49^{cC}±0.01	107.49^{cA}±1.28
	NOV2300	115.14^{cA}±3.41	125.33^{cB}±1.64	121.68^{cAB}±1.62	122.18^{cAB}±0.17
	CAR	166.08^{dA}±0.10	210.74^{dB}±3.44	151.71^{dA}±0.48	193.32^{dA}±7.53

* Means with different lower case letters differ significantly in the vertical ($p < 0.05$).
** Means with capital letters horizontally differ significantly ($p < 0.05$).
*** The acronyms correspond to: (SPI) soy protein isolate; (TSP1); texturized soy protein 1, (TSP2); texturized soy protein 2; (GG) guar gum; (CSP) concentrated soy protein; (CAR) kappa-carrageenan; (ULTRATEX8) Ultratex-8 modified starch; (NOV1900) Novation 1900 modified starch; (MS) Eliane 100 modified starch; (THERM) Thermetex modified starch; (NOV2300) Novation 2300/EK 8925 modified starch; (CS) cassava starch; and (ULTRATEX) Ultratex 3 modified starch

cleaning laboratory parts and materials (UNIQUE, SC1600, model 3.8 L, Indaiatuba, SP, Brazil). The power used was 135 W and the frequency was 40 k Hz.

Three repetitions were performed for each experiment and the analyses were carried out in triplicate, at least. The results were submitted to analysis of variance (ANOVA) and Tukey's test, with a significance level of 95% ($p < 0.05$), using Statistica® 9.0 (StatSoft Inc., Tulsa, OK, USA) software. The graphics and calculations of the effects were also obtained using the aforementioned computer program and Microsoft® Excel 2003 (Microsoft Brazil, São Paulo, SP, Brazil) software. Only models that were considered to be adequate are presented in this article ($R^2 > 0.90$, and in the ANOVA table the value of F calculated for the regression was greater than the tabulated values; the value of F calculated for the residue was lower).

Results and Discussion

Table 2 shows the results obtained for the WAC (%) for each ingredient or additive that was evaluated in the different saline solutions (2, 4, 6 and 8%). Analyzing the data shown in Table 2, it can be seen that there was a significant difference ($p < 0.05$) in the WAC values between the tested ingredients and additives, and that there was also significant difference ($p < 0.05$) when compared to the saline concentrations, except in the case of the SPI sample, which showed no significant variation in WAC with increased saline concentration in the added solution. It was found that the modified starches MS, THERM and the cassava starch (CS) showed the lowest WAC values, and that there was also a significant difference ($p < 0.05$) for the different proteins of the tested soya. The low levels of WAC for the CS sample can be explained because its granules are insoluble and hydrate very little in cold water (Damoradan *et al*., 2010).

Analyzing the proteins, the highest WAC values were found for the CSP sample in an 8% saline concentration (368.26%). This result can be explained by the increased surface area caused by the finer particles (dust) in relation to the other types of protein (flakes or granules) and also because of the higher solubility of this protein due to the industrial methods of extraction and purification. The TSP1 and TSP2 protein samples differed in WAC and this difference can be explained by particle size, protein content and possible variations in the process by which they were obtained by the manufacturers.

Among the polysaccharides, the highest WAC

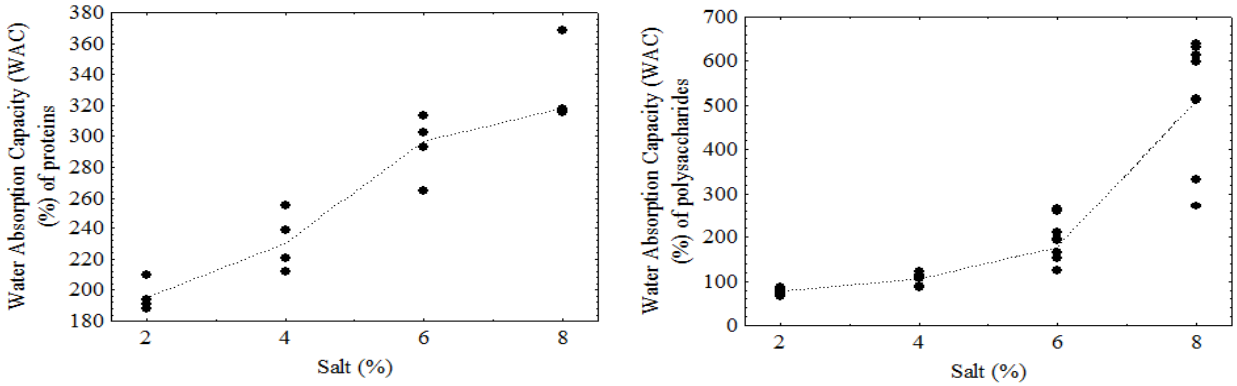

Figure 1. (a) Average water absorption capacity (%) for the group of proteins in relation to the % of added salt. (b) Average water absorption capacity (%) for the group of polysaccharides in relation to the % of added salt.

value was observed for the GG (639.97%) and ULTRATEX3 (515.32%) samples in 8% saline concentration. This high WAC value can be explained by a greater interaction between the charged residues and ions derived from the dissociation of the salt; when these are present in solution they provide a more extended chain which favors solubility, as suggested by Aranda-Selverio *et al.* (2010).

The average WAC of the two groups in relation to increases in saline in the added solution is shown in Figure 1. It can be seen that, on average, for both groups there was an increase in WAC, with the highest values observed for the polysaccharides (about 1.7 times greater than the WAC found for the proteins). The addition of salts to polysaccharide solutions can promote changes, increasing or decreasing the solubility. The variation in the degree of substituent of a polysaccharide changes its rheological properties in solution and this behavior can be attributed to the interaction between charged residues and ions that result from the dissociation of the 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 (number of charges per unit of length) modifies the macromolecular properties in solution (Aranda-Selverio *et al.*, 2010). Ramaswamy *et al.* (2013) reported that linear arabinan chains showed greater water retaining capacity than branched chains, which can be explained by the higher mobility of the molecules in solution, which allows greater interaction with water.

In terms of proteins, the addition of salt can lead to the approximation of the IP (isoelectric point) and this alters solubility because it modifies the distribution of hydrophobic and hydrophilic amino acids on the surface (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. In certain concentrations, sodium chloride enhances the solubility of proteins. A mechanism called 'salting in' occurs, in which the charged groups on the surface attract and bind anions and cations more strongly than they do with water. However, these ions bring with them an ordered grouping of their own molecules of solvation water, 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 the salt ions and the protein by the water required to maintain the protein in solution, so that the salt concentration rises greatly as the proteins precipitate (Coultate, 2004).

The second stage of the experiments involved the addition (or not) of 2% saline solution and the application of ultrasound for 15 or 30 minutes for the thirteen ingredients/additives that were tested. It was decided to use 2% saline solution because this is the concentration that is most widely used for meat products by the industry, and also for a better visualization of the effects of ultrasound on the samples that were tested. The WAC results for the samples subjected to ultrasound are shown in Table 3.

It was observed that there was significant difference ($p < 0.05$) in the WAC values (%) between the tested ingredients and additives for the treatments TRT1, TRT2, TRT3 and TRT4. Among the proteins, only the TSP2 sample did not show a significant difference in the WAC between the different treatments. However, for the polysaccharides there was no significant difference ($p > 0.05$), between the treatments for the samples THERM, ULTRATEX3, MS, NOV1900, NOV2300 and CS. Only the ULTRATEX8, CAR and GG samples showed changes in WAC with the addition of saline solution and/or the application of ultrasound.

The modified starches MS, THERM and cassava starch (CS) showed the lowest WAC values and that

Table 3. Water absorption capacity (%)for the evaluated additives or ingredients (proteins and polysaccharides) in different saline concentrations and subjected to different times in an ultrasound.

Groups/Treatments		TRT1 (0%, 15 min)	TRT2 (0%, 30 min)	TRT3 (2%, 15 min)	TRT4 (2%, 30 min)
Proteins	SPI	368.84^{dB}±0.61	357.73^{cdB}±0.76	318.95^{dA}±5.04	303.57^{dA}±0.24
	TSP1	200.82^{bAB}±3.78	210.88^{bB}±0.58	195.19^{cA}±1.37	194.38^{bA}±2.12
	TSP2	286.17^{cA}±0.51	278.72^{bcA}±1.40	279.81^{dA}±0.91	283.56^{cdA}±1.82
	CSP	400.62^{deC}±1.28	404.38^{dC}±1.10	320.43^{dA}±3.29	339.93^{dB}±0.43
Polysaccharides	THERM	86.04^{aA}±0.68	87.58^{aA}±0.26	87.61^{abA}±1.10	88.02^{aA}±0.95
	ULTRATEX8	394.39^{dA}±1.23	521.76^{eBC}±4.22	606.43^{fC}±8.12	574.51^{eBC}±0.35
	ULTRATEX3	480.09^{eA}±4.93	574.85^{eA}±0.14	521.14^{eA}±5.52	557.73^{eA}±4.90
	MS	78.74^{aA}±1.20	81.36^{aA}±0.84	82.41^{abA}±0.45	81.33^{aA}±2.00
	NOV1900	106.03^{aA}±0.71	108.42^{aA}±0.53	97.44^{abA}±5.14	100.60^{aA}±0.07
	NOV2300	112.06^{aA}±3.53	115.02^{aA}±0.20	122.05^{bA}±0.59	117.54^{aA}±2.38
	CAR	225.13^{bcAB}±3.40	254.45^{bC}±0.58	208.63^{cA}±0.33	244.30^{bcBC}±1.70
	GG	597.69^{fAB}±2.88	595.76^{eA}±0.14	607.62^{fB}±2.49	607.23^{eAB}±0.66

* Means with different lower case letters differ significantly in the vertical ($p < 0.05$).
** Means with capital letters horizontally differ significantly ($p < 0.05$).
*** The acronyms correspond to: (SPI) soy protein isolate, (TSP1); texturized soy protein 1, (TSP2); texturized soy protein 2; (GG) guar gum; (CSP) concentrated soy protein; (CAR) kappa-carrageenan; (ULTRATEX8) Ultratex-8 modified starch; (NOV1900) Novation 1900 modified starch; (MS) Eliane 100 modified starch; (THERM) Thermetex modified starch; (NOV2300) Novation 2300/EK 8925 modified starch; (CS) cassava starch; and (ULTRATEX) Ultratex 3 modified starch.

there was also a significant difference ($p < 0.05$) for the different soy proteins that were tested, with the highest WAC value for the CSP sample. Comparing Treatment 1 (0% NaCl, 15 min) with Treatment 2 (0% NaCl, 30 min) it was noted that there was an increase in WAC for most of the ingredients or additives that were tested, which was in line with increased time of exposure to ultrasound. Comparing Treatment 3 (2% NaCl, 15 min) and Treatment 4 (2% NaCl, 30 min) with Treatment 1 (0% NaCl, 15 min) and Treatment 2 (0% NaCl, 30 min) it was noted that the addition of salt caused no increase in WAC for most samples, with the exception of ULTRATEX8, which presented the highest value.

According to Hu *et al.* (2013), the use of ultrasound can lead to the formation of highly reactive free radicals due to breakage of the water molecules, and these radicals can react with other molecules, which may account for the increased absorption of water of Treatment 2 in relation to Treatment 1. This behavior was most pronounced for the group of polysaccharides.

Among the group of proteins, the CSP sample showed the highest WAC, which can be explained by the greater presence of polysaccharides in this type of protein in relation to the SPI sample. The level of polysaccharides in the CSP sample was about 30%, whereas in the SPI sample it was approximately 5%. Although Treatment 1 and Treatment 2 presented about 50% of polysaccharides in their composition, it is possible that due to the larger particle size of these proteins the effects of ultrasound were not the same as was obtained for the CSP sample, which was in powder form (a larger surface area available for hydration). According to Wu *et al.* (2011) smaller particle size increases solubility and water retention.

Hu *et al.* (2013) reported increased water absorption for soy protein isolate treated with ultrasound (20 k Hz, 400 W) at different times. At a time of 20 min they observed higher values of water absorption (95.53%) than at 40 min (90.50%). According to these authors, increased exposure to ultrasound may cause denaturation of the protein structure, which reduces the capacity for water retention. A reduction in WAC caused by longer periods of exposure to ultrasound can also be justified by the greater exposure of hydrophobic regions present in the protein structure, due to the phenomenon of cavitation.

Figure 2 show the WAC relative to the two groups (proteins and polysaccharides). It can be seen that the SPI and CSP protein samples (which were in powder form) showed higher WAC values and that these results were observed for Treatments 1 and 2.

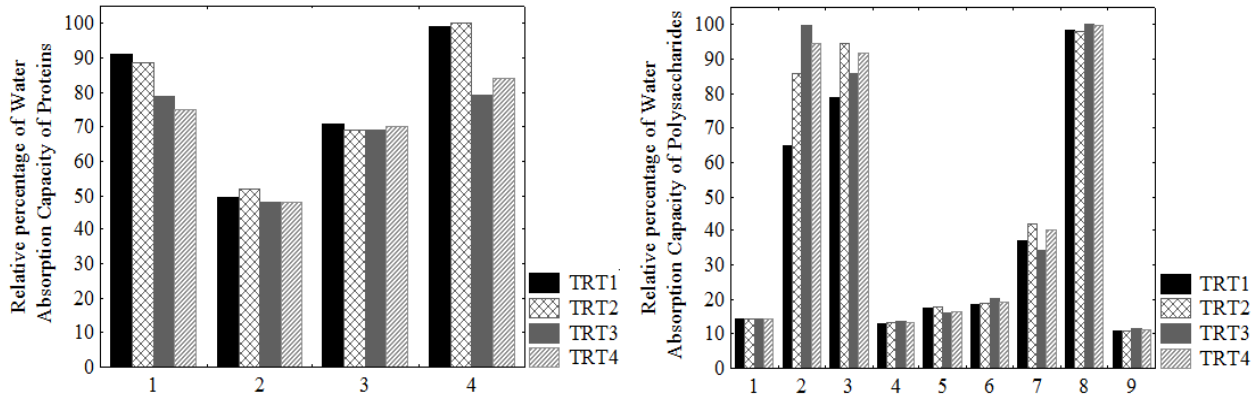

Figure 2. (a) Relative water absorption capacity (%) for the tested group of proteins in relation to the 2² factorial design treatments. *1 corresponds to (SPI) soy protein isolate; 2 corresponds to (TSP1) texturized soy protein 1; 3 corresponds to (TSP2) texturized soy protein 2; and 4 corresponds to (CSP) concentrated soy protein.(b) Relative water absorption capacity (%) for the tested group of polysaccharides in relation to the 2² factorial design treatments.**1 corresponds to (THERM) Thermetex modified starch; 2 corresponds to (ULTRATEX8) Ultratex-8; 3 corresponds to (ULTRATEX3) Ultratex 3 modified starch; 4 corresponds to (MS) Eliane 100 modified starch; 5 corresponds to (NOV2300) Novation 2300/EK 8925 modified starch; 6 corresponds to (NOV1900) Novation 1900 modified starch; 7 corresponds to (CAR) kappa-carrageenan; 8 corresponds to (GG) guar gum; and 9 corresponds to (CS) cassava starch. *** TRT 1 corresponds to 0% NaCl and 15 min; TRT 2 corresponds to 0% NaCl and 30 min; TRT 3 corresponds to 2% NaCl and 15 min; and TRT4 corresponds to 2% NaCl and 30 min.

It can also be seen that the particle size, and hence the surface area, directly influenced the WAC. For the group of polysaccharides, the behavior was very similar to that of the modified starches (ULTRATEX3 and ULTRATEX8) and CAR, which showed the highest WAC values, and the addition of salt and treatment with ultrasound increased absorption (Treatments 3 and 4).

The values of the effects that were significant ($R^2 > 0.70$) (results not shown). First order effects were only observed for the SPI, CAR and CSP samples. Regarding the SPI sample, the increase in time of exposure to ultrasound produced a significantly negative effect on the WAC. For the CAR sample, the time effect was positive and increased the WAC. On the other hand, for the CSP sample there was a significant positive effect regarding the time, and a negative reaction regarding the saline concentration. Although it was expected, none of the ingredients or additives that were tested had a significant effect regarding the interaction of time of exposure to ultrasound and saline concentration.

Regarding the effects of ultrasound on polysaccharides, some authors (Enbrigerová *et al.*, 1997) have suggested that sonication affects their structure, which leads to changes in viscosity and higher solubility in water. Jambrak *et al.* (2010) studied the effect of ultrasound (bath and probe) on the properties of corn starch (5 or 10%) with time intervals of 15 or 30 min. The results indicated changes in the crystalline region of the starch granules, increased solubility, and higher water absorption, as well as swelling, which directly affected gelatinization. Czechowska-Biskup *et al.* (2005) also reported a reduction in molecular weight and the degradation of polysaccharides (starch and chitosan), which caused the formation of radicals and the alteration of mechanical properties.

Under the tested conditions, ultrasound proved to be an interesting alternative for companies producing additives and ingredients, due to increased water absorption, mainly for the polysaccharides that were evaluated. Of particular note was the increased absorption found for the GG sample and the ULTRATEX3 and ULTRATEX8 modified starches. In relation to the proteins, ultrasound did not favorably affect WAC, which was probably due to the increased exposure of hydrophobic radicals and also due to protein denaturation. The type of protein, the particle size, and the manner in which it was obtained by the manufacturing industry also directly influenced WAC and the effects produced by ultrasound.

Conclusion

There was significant variation ($p < 0.05$) in WAC (%) for the different groups of additives and ingredients that were tested. The highest WAC (%) was found for guar gum (639.96% to 8% salt) and the lowest was for cassava starch (67.08% to 2% salt). Both groups showed an increase in WAC in line with an increase in salt concentration in the solution that was added. For the treatments with ultrasound,

there was an increase in WAC for most of the tested polysaccharides. Comparing the treatments using the 2^2 factorial design, Treatment 3 (2% NaCl, 15 min) and Treatment 4 (2% NaCl, 30 min) with Treatment 1 (0% NaCl, 15 min) and Treatment 2 (0% NaCl, 30 min), it was observed that the addition of salt, concomitantly with the use of ultrasound, increased the WAC of only one of the thirteen samples that were evaluated.

These results can help in the development of new formulations of meat products on an industrial level by reducing the testing time and facilitating the choice of the best or most appropriate ingredient/additive, according to the application and/or saline concentration in the final product. Under the tested conditions, ultrasound proved to be an interesting alternative for companies producing additives and ingredients due to the increased water absorption, mainly for the polysaccharides that were evaluated.

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