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CRYOPROTECTION OF PORK

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Key words: cryoprotectants, polydextrose, carrageen, pork, freezing, cryoprotection, protein solubility, glass transition

The aim of the study was to determine the effect of polydextrose (6% addition) and a mixture of polydextrose (4%) with carrageen (0.2%) on the functional properties and thermostability of pork stored for 12 weeks at a temperature -26°C. It was proved that cryoprotectants could be used for the protection of the functional properties of meat. Cryoprotectants can be added to meat in the form of injected solutions and suspensions. The addition of polydextrose and its mixture with carrageen elevates glass transition temperature, inhibits a decrease in soluble protein content as well as increases and stabilises water holding capacity. The addition of 6% polydextrose to meat increases significantly the temperature of thermal myosin denaturation. The results obtained confirm the theory proposed by Carpenter and Crowe that explains the mechanism of cryoprotective action of compounds with high molecular weight.

INTRODUCTION

Negative changes in meet during frozen storage can be identified with changes in the physicochemical properties of myofibrilar proteins [Park & Lanier, 1993]. This statement is true also because among all muscle proteins, myofibrilar proteins are characterised by the highest sensitivity to freeze damage. These changes can be reduced remarkably by adding the so-called "cryoprotectants" to meat before freezing. The favourable action of cryoprotectants was documented in reference to myofibrilar protein isolates and surimi-type preparations from fish and poultry meat [Park & Lanier, 1993; Yoon & Lee, 1990; Yang *et al.,* 1990; Simpson &Morrisey, 1995; Kijowski & Richardson, 1996].

There are only a few publications on cryoprotection of meat proteins of slaughter animals and poultry. The application of cryoprotectants in the technology of freeze protection of meat seems to be fully justified. Kijowski & Richardson [1996] in their studies into the effect of cryoprotectants on the mechanical properties of recovered broiler meat (MOM), proved that the addition of saccharose and sorbitol mixture with STP reduced the negative changes in frozen meat functionality. Although results of these studies are very promising, the applicability of saccharose and sorbitol in the technology of frozen stored beef and pork is very unlikely since saccharose adds sweet taste to the preserved product, and sorbitol is several times more expensive than meat itself. Hence, the use of this additive would have an influence on the price of the final product. Park & Lanier [1993] showed that a similar effect was evoked by the application of much cheaper polydextrose. They proved that the addition of 8% polydextrose to minced beef inhibited a decrease in muscular protein solubility during storage at -28°C.

Recently, much interest of researchers dealing with cryoprotection of meat has been focused on hydrocolloids. They are an important group of texture-forming food components or food additives [Makała, 2003; Adamczak *et al.*, 2003a,b]. The cryoprotective properties of protein and saccharide hydrocolloids have been tested at the Technology of Food Refrigeration Unit, Łódź Technical University, [Dziomdziora & Krala, 2000, 2003]. The aim of the research was to determine the effect of cryoprotectants on technological properties of pork stored at a temperature of -26° for 12 weeks. It was shown that among several analysed cryoprotectants, the most efficient in improving the functional properties of frozen minced meat were 1.5% protein isolate of bovine blood plasma and 0.4% sodium alginate or carrageen. It was observed that the stability of frozen products could be obtained by getting them to the glassy state.

Glass transition is a process in which water is not crystallised but is in an amorphous form in the system. Such a system reaches the viscosity of 10^{12} Pa·s. In the glassy state, all physical and chemical processes are inhibited. Due to this, food products can be stored for a long time without the risk of deterioration. The glass transition, or strictly speaking temperature of this process T_g, is one of the basic parameters that characterise the quality of a dehydrated (frozen or dried) product. It has been proved recently that T_g has a major influence on the stability of food components and food products [Li & Chien, 2001; Bell & White, 2000]. An increase in T_g to the values applied in the technology of frozen preservation of water would allow meat to retain the technological utility for a long time.

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The scope of research published so far has not addressed the possibility of using cryoprotectants to not comminuted meat of slaughter animals. The reason seems to be the physicochemical properties of cryoprotectants themselves. These compounds do not diffuse into muscle tissue. Thus, to intensify their action, cryoprotectants are added to the comminuted muscle tissue.

The aim of the research was to determine the effect of polydextrose and its mixture with carrageen on the functional properties and thermostability of pork during frozen storage.

MATERIALS AND METHODS

The tested material was pork in the form of whole hams separated from semi-carcasses immediately after cooling. The tested cryoprotectants included polydextrose, added at a dose of 6% (**samples C1**) and a mixture of polydextrose (4%) and carrageen E407 (0.2%) (**samples C2**). The cryoprotectants were added to meat in the form of solutions and suspensions. They were injected uniformly into the whole boned ham and its weight was controlled. To equalise the concentrations of additives, the hams after injection were conditioned for 12 h at a temperature of 0–2°C. After that time, muscles were separated from bones, fat and skin and cut into cubes of sides *ca.* 3 cm, mixed, portioned and packed into shrink film bags. The bags were closed by heat sealing. The packed samples were frozen to a temperature of -26°C and stored at this temperature for 12 weeks. Control samples in this experimental series were similarly the pieces of mixed ham muscles, however, without cryoprotectants (samples K). After specified storage time, the samples were taken for analyses. Samples selected at random were thawed at 0–2°C for 12 h and then mixed.

To determine the effect of applied cryoprotectant on thawed meat properties, the samples were determined for: the content of soluble proteins – the proteins were extracted by Dyer's method, the content of proteins in the extract was determined by Lawry's method [Krełowska-Kułas, 1993]; water holding capacity (WHC) by the modified Grau-Hamm method [Krala, 1995]; temperature of thermal protein denaturation by the differential scanning calorimetry (DSC) and glass transition temperature by the DSC method; electrophoresis SDS-Page.

Significant differences between arithmetic means of results from particular experimental series were determined based on one-way analysis of variance (Snedecor F-test) and statistical functions calculated according to t-Student test (α=0.05). An Excel calculation sheet with Analysis ToolPak and Solver was used in the calculations.

RESULTS AND DISCUSSION

Table 1 compiles the results of analyses into the content of soluble proteins in the tested meat. It was found that polydextrose had a favourable effect on soluble protein content over 4 weeks of frozen storage of meat. Both in the samples containing 6% polydextrose and in the meat with 4% polydextrose addition, no statistically significant differences were found in soluble protein content over the entire frozen storage period. The addition of carrageen to meat had no significant effect on the values of this indicator of meat technological utility.

TABLE 1. Soluble protein content of pork frozen with the additives: C1 – polydextrose (6%), C2 – polydextrose (4%) and carrageen (0.2%). Symbol K denotes meat without additives.

Time (weeks)	Soluble protein content ± SD (% total protein) n=15		
	K	C1	C2
0	$16.1^{a1} \pm 0.9$	$15.8^{a1} \pm 0.9$	$15.8^{a1} \pm 0.9$
1	$13.9^{b1} \pm 0.4$	$14.8^{b2} \pm 0.3$	$14.9^{b2} \pm 0.5$
4	$13.2^{bc1} \pm 0.4$	$14.0^{bc2} \pm 0.8$	$14.1^{bc2} \pm 0.5$
8	$12.8^{cd1} \pm 0.7$	$13.2^{cd1} \pm 0.7$	$13.3^{cd1} \pm 0.6$
12	$12.3^{d1} \pm 0.8$	$12.6^{d1} \pm 0.7$	$12.5^{d1} \pm 0.4$

values denoted by the same letter superscripts within one column do not differ significantly ($p < 0.05$); values denoted by the same digital superscripts within one row do not differ significantly ($p < 0.05$)

Water holding capacity of meat with the addition of 6% polydextrose was higher in view of statistical significance during the entire frozen storage period than in the meat without additives (Figure 1). The addition of a mixture of carrageen and polydextrose enhanced a positive effect of these agents, which can be an evidence of their synergetic interaction on WHC of frozen meat. In samples with the addition of this mixture before freezing, as well as during frozen storage, no statistically significant changes of WHC were reported. WHC of this meat was higher as compared to other samples. An increase in WHC of fresh meat and its products upon the addition of hydrocolloids and protein isolates was also a subject of other researches [Adamczak *et al.*, 2003a, b]. They showed that hydrocolloids (carrageen, alginate) improved remarkably WHC of minced meat. Their addition reduced mass losses during thermal processing and increased holding capacity of water added during the production process. The effect of carrageen and protein hydrolysate on protein denaturation in frozen surimi was investigated by Sych & Lacroix [1990a, b]. The authors explained the improved WHC of surimi with the addition of carrageen and protein isolate by the fact that they formed gel structures in free spaces of the product. Dziomdziora & Krala [2000, 2003] proved that the addition of hydrocolloids to minced pork improved remarkably and stabilised WHC during frozen storage.

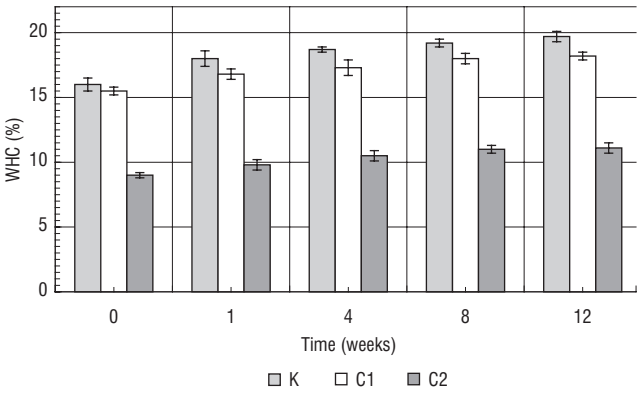

FIGURE 1. WHC of pork frozen with additives: C1 – polydextrose (6%), C2 – polydextrose (4%) and carrageen (0.2%), K – meat without additives (n = 15).

Thermal denaturation of proteins is represented by endothermal peaks in the thermograms of differential scanning calorimetry. Thermal denaturation processes can be interpreted by maximum temperature values (T_{max}) of subsequent endothermal peaks [Stabursvik & Martens, 1980]. The differential scanning calorimetry enables determination of the temperature of thermal protein denaturation in muscle tissues and in muscle protein extracts. In the thermogram we obtained (Figure 2), there are four endothermal peaks. The origin of the first one with T_{max} from 28.92°C for meat with a 6% polydextrose addition to 32.55°C for meat without additives, is difficult to interpret. Park & Lanier [1987b], in their studies on thermal denaturation of pre-rigor and post-rigor beef obtained four endothermal peaks on DSC thermograms, at T_{max} of the first one ranging from 35°C to 39°C. They demonstrated also that the interpretation of this peak origin is not clear. It is supposed to correspond to the denaturation of the most thermolabile muscle proteins or the transformations of meat components. According to Kijowski & Mast [1988a, b], the peak of this temperature range corresponds to changes in the lipid fractions in meat. From the point of view of meat technological utility, the most interesting are the three subsequent peaks which represent temperatures of thermal denaturation of myofibrilar and sarcoplasmatic proteins. Maximum temperatures of the second peak on the discussed thermograms (Figure 2) are 57.30°C for sample C1, 55.10°C for sample C2 and 55.11°C for the control sample, respectively. Numerous studies on thermal denaturation of myofibrilar proteins in various kinds of meat [Park & Lanier, 1987b; Stabursvik & Martens, 1980; Kijowski & Mast 1988a,b; Findlay & Stanley, 1984a,b] have proved that this T_{max} range corresponds to thermal denaturation of myosin. In complex systems, such as meat, thermal denaturation of myosin is usually illustrated by one peak in the T_{max} range from 48°C to 57C. According to the quoted authors, divergence of T_{max} values results from dissimilarities between animal species. The least resistant to changes in thermal denaturation are fish proteins. In DSC thermograms of thermal denaturation of myofibrilar protein isolates, one can observe a number of peaks illustrating denaturation of particular myosin fractions [Park & Lanier, 1987b].

The results of our study (Figure 2) indicate that a 6% polydextrose addition to meat greatly affected the elevation of temperature of thermal denaturation of bovine myosin. The authors showed also that frozen storage had no effect on the temperature of thermal denaturation of myosin. In beef, prior to freezing as well as after 5 months of frozen storage, no temperature difference was found in thermal denaturation of myosin. These authors obtained similar results when they were analysing temperature changes in thermal denaturation of actin [Park & Lanier, 1987b]. In DSC thermograms, the denaturation of meat actin is represented by the peak of the highest T_{max}. In this thermogram (Figure 2), actin denaturation is illustrated by the fourth peak for which T_{max} was 77.39°C for sample C1, 77.47°C for sample C2 and 78.23°C for sample K. These temperatures correspond to the temperature ranges of thermal denaturation of actin shown by many researchers [Park & Lanier, 1987b; Stabursvik & Martens, 1980; Kijowski & Mast, 1988a, b; Findlay & Stanley, 1984a, b]. The results showed that the addition of polydextrose had no significant effect on the temperature of thermal denaturation of pork actin. Park & Lanier [1987b] did not find any important effect of polydextrose on temperature changes in thermal denaturation of bovine actin either. The third peak in the discussed DSC thermogram corresponds to thermal denaturation of sarcoplasmatic proteins and collagen (Figure 2). Temperatures T_{max} for this peak are 61.76° (C1), 63.36°C (C2) and 64.66°C (K). It follows from results obtained that the addition of 6% polydextrose reduces remarkably the temperature of thermal denaturation of sarcoplasmatic proteins. To verify this observation further studies are needed.

In studies on glass transition temperature (Figure 3), we proved that the addition of polydextrose and its mixture with carrageen had a significant influence on the increase of

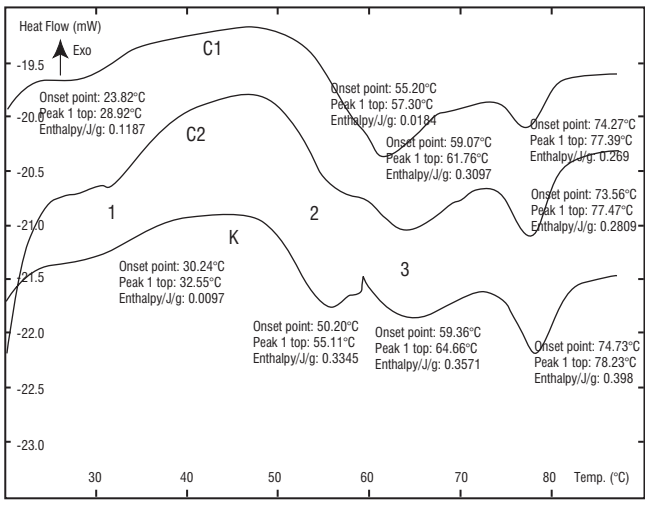

FIGURE 2. DSC thermogram of pork with the addition of cryoprotectants: C1 – meat with polydextrose (6%), C2 – meat with polydextrose (4%) and carrageen (0.2%), K – meat without additives.
1 onset point: 28.90°C, Peak 1 top: 30.67°C, Enthalpy / J/g: 0.0348
2 onset point: 51.33°C, Peak 1 top: 55.1°C, Enthalpy / J/g: 0.0872
3 onset point: 58.88°C, Peak 1 top: 63.36°C, Enthalpy / J/g: 0.2338

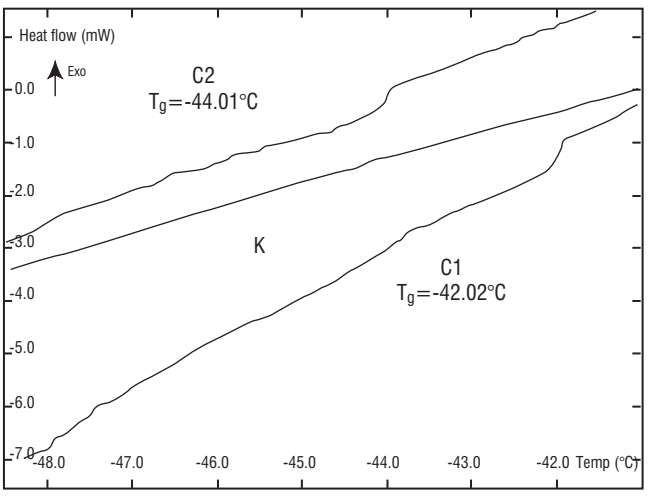

FIGURE 3. Glass transition temperatures of pork with cryoprotectants: C1 – meat with polydextrose (6%), C2 – meat with polydextrose (4%) and carrageen (0.2%), K – meat without cryoprotectants.

glass transition temperature in pork. In meat without additives, no glass transition was observed because it exceeded the range of temperature measurements. Inflections of the temperature curve characteristic of the glass transition were reported in the samples with the addition of tested cryoprotectants. For meat with the addition of 6% polydextrose, the glass transition temperature T_g was -42.02°C, whereas for the samples with 4% polydextrose and 0.2% carrageen it was -44.01°C. These results lead to a conclusion that polydextrose added to pork hightly increases the glass transition temperature.

The results obtained in our experiments seem to confirm the mechanism of cryoprotective action of polysaccharides with high molecular weight, as suggested by Carpenter and Crowe [MacDonal & Lanier, 1991]. According to these authors, cryoprotectants with high molecular weight increase T_g of frozen systems. Numerous studies of T_g in model solutions of oligo- and polysaccharides [Ross, 1995; Bell & Toma, 1996; Levine & Slade, 1990] have proved that their type and concentration had a great effect on glass transition temperature. Levine & Slade [1990] proved that T_g of a 10% solution of maltodextrin 10DE was -9°C, whereas of 20% saccharose solution -21°C, which suggests that according to the theory proposed by Carpenter and Crowe [MacDonal & Lanier, 1991] polysaccharides with high molecular weight increase the glass transition temperature to a greater extent.

The results of SDS electrophoresis (Figure 4) revealed that cryoprotectants applied in the tests had a protective action on proteins during frozen storage of meat. In particular, this effect was tangible in the myosin fraction (205 kD). After four weeks of frozen storage, in the samples with 6% polydextrose (C1) and 4% polydextrose mixed with 0.2% carrageen (C2), the presence of myosin (205 kD) was reported. In the control sample, after the same period of frozen storage myosin was presented in a vestigial amount. Our results are in agreement with the results obtained by Park and coworkers [Park *et al.*, 1987a, b]. The authors confirmed a protective action of polydextrose on myosin in fish. According to Park [1993; 1994a, b], this protective effect of cryoprotectants on meat is not limited to frozen storage only. It can be expected that cryoprotectants protect also proteins in meat subjected to thermal processing. Results of experiments carried out within this research confirm this assumption. It was shown that polydextrose increases the temperature of thermal denaturation of myosin in pork and also protects this protein during frozen storage of meat.

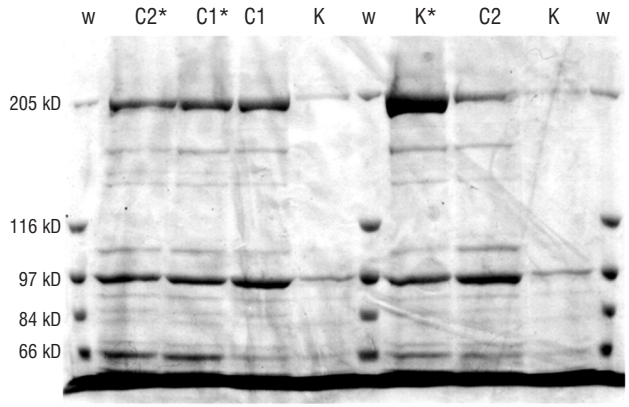

FIGURE 4. SDS of proteins in pork frozen with addition of polydextrose (6%) – C1, polydextrose (4%) and carrageen (0.2%) – C2. Symbol K denotes meat without cryoprotectants. Index * denotes samples before freezing. Other samples were analysed after 4 weeks of frozen storage at a temperature -26°C.

CONCLUSION

The addition of polydextrose and its mixture with hydrocolloids to meat before freezing counteracts negative changes in its functional properties and physicochemical characteristics during frozen storage. Hence, cryoprotection of pork is possible.

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KRIOPROTEKCJA MIĘSA WIEPRZOWEGO

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Celem badań było określenie wpływu oddziaływania polidekstrozy (dodatek 6%) i mieszaniny polidelstrozy (4%) z karaganem (0,2%) na właściwości funkcjonalne i termostabilnośc nierozdrobnionego mięsa wieprzowego przechowywanego w temperaturze -26°C przez okres 12 tygodni. Wykazano, iż możliwe jest stosowanie krioprotektantów do ochrony właściwości funkcjonalnych mięsa nierozdrobnionego. Krioprotektanty można dodawać do takiego mięsa w postaci roztworów i zawiesin metodą nastrzyku. Dodatek do mięsa polidekstrozy i jej mieszaniny z karagenem podwyższa temperaturę przejścia szklistego spowalnia spadek zawartości białek rozpuszczalnych oraz podwyższa i stabilizuje wodochłonność. Dodatek 6% polidekstrozy do mięsa znacząco podwyższa temperaturę cieplnej denaturacji miozyny. Uzyskane wyniki potwierdzają teorię Carpentera i Crowa, wyjaśniającą mechanizm krioprotekcyjnego działania związków o dużej masie molowej.