Inhibition of Formaldehyde Production in Frozen-Stored Minced Blue Whiting (*Micromesistius poutassou*) Muscle by Cryostabilizers: An Approach from the Glassy State Theory

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Adding maltodextrins to minced blue whiting muscle inhibited formaldehyde production during storage at −10 and −20 °C. Sucrose, however, was effective only at −20 °C. These results were not proportional to the difference between the storage temperature and the ice-melting onset (T_m'), and freeze−concentration had to be considered. In the face of serious limitations to do this, resource was made to the state diagram for the sucrose−water binary system and the percentage variation of the ice-melting endotherm area of the different samples. A higher T_m' and a lower freeze−concentration would account for the inhibiting effects of maltodextrins, whereas sucrose, despite diminishing T_m', had an effect nearly as great as maltodextrins at −20 °C but had hardly any at −10 °C. The reason for this seems to lie in a lower freeze−concentration of solutes in the unfrozen water phase. Similarly, the differences found between sucrose and maltodextrins, as well as among maltodextrins, were also explained in terms of T_m' and freeze−concentration.

Keywords: *Minced fish muscle; formaldehyde production; cryostabilization; ice-melting onset; glass transition temperature; freeze−concentration; unfrozen water; maltodextrins; sucrose; state diagrams*

INTRODUCTION

Formaldehyde (FA) production is considered to hasten the loss of protein functionality and the textural deterioration of gadoid muscle (Gill et al., 1979; Hultin, 1992). Although mincing favors the production of FA and its subsequent reactivity (Hiltz et al., 1976; Parkin and Hultin, 1982), it makes possible the use of underutilized species, scraps resulting from filleting, and wastes that otherwise would be discarded. This, along with overfishing of most commercial species, has made the use of minced fish increase (Pastoriza et al., 1994; Borderías and Pérez Mateos, 1996).

Various alternatives have been proposed to prevent FA production as this would be expected to enhance the stability of frozen fish (Lall et al., 1975; Kelleher et al., 1981; Landolt and Hultin, 1982; Parkin and Hultin, 1982; Reece, 1985). In this respect, the addition of cryoprotectants such as sucrose, sorbitol, or polydextrose has been proved to reduce the levels of FA produced (MacDonald et al., 1990; Herrera and Mackie, 1994; Simpson et al., 1994). Nonetheless, recent studies have shown that sucrose was effective during storage at −20 °C, but not at −10 °C, whereas a number of maltodextrins showed a noticeable inhibiting effect at those two storage temperatures, which, however, was temperature-dependent, too (Herrera et al., 1999).

The food polymer science approach states that the stability of a frozen food is determined by the location of the glass transition of the freeze−concentrated unfrozen water (UFW) phase. This transition defines whether the UFW phase is in a glassy or a rubbery state. Theoretically, the glass transition temperature, T_g, also marks the onset of ice melting, but glass transitions usually occur over a temperature range, so T_g and ice-melting onset may be different (Roos and Karel, 1991a; Simatos and Blond, 1991, 1993). When freeze−concentration is maximum, T_g is designated T_g', and the onset of ice melting is named T_m'. Storage at $T < T_g'$ has been considered to be necessary for a frozen system to be stabilized (Levine and Slade, 1988, 1990). However, frozen storage temperatures are usually higher than T_g' (and T_m') so food systems are unstable and highly reactive. Nevertheless, T_g' (and also T_m') can be increased by adding polymeric cryostabilizers such as maltodextrins, which thus would prevent or slow diffusion-controlled processes. Accordingly, the inhibitive effect of maltodextrins on FA production was in principle attributed to a restriction of molecular diffusion (Herrera et al., 1999).

The present study has therefore focused on the applicability of the principles of the glassy state theory to the inhibitive effect of maltodextrins and sucrose on the production of FA in minced blue whiting muscle during storage in the frozen state.

MATERIALS AND METHODS

Preparation of Samples. Blue whiting fish (*Micromesistius poutassou*), caught in off-shore Galician waters, were brought layered between crushed ice in polyfoam boxes to the laboratory within the first 24 h postcapture. Fish were immediately gutted and beheaded, and fillets were taken off and skinned. Muscle was then minced in a meat mincer (Cutter DITO SAMA K-35) and subsequently mixed manually with a number of maltodextrins of different dextrose equivalents (DE 4, 9, 12, 18, and 28) (Cerestar Ibérica, S.A.) or with sucrose at a concentration of 8% (w/w). The notations used for the samples correspond to the cryostabilizer added, for example, MD DE 9 for the sample with maltodextrin of dextrose equivalent 9. A batch with no additive was also used as a control.

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Samples (~30 g, 0.5−0.8 cm thick) were placed in individual plastic bags, which were sealed and frozen in a freezer cabinet set at −20 °C. After overnight storage, half of the samples were transferred to a freezer cabinet set at −10 °C, and the remaining half was kept at −20 °C. Samples stored at each temperature were periodically taken out of the freezers, and their formaldehyde contents were determined.

Initial Analyses: Moisture and Water-Soluble Solute Contents. Both moisture and water-soluble solute contents were initially determined in nonfrozen blue whiting muscle (triplicate samples). These contents were used to estimate the concentration of fish components in the unfrozen water phase in both control and sucrose-containing samples at each storage temperature.

The moisture content was determined by gravimetry after minced fish had been dried to a constant weight at 105 °C.

The content of water-soluble solutes was gravimetrically determined by drying water-soluble fish muscle extracts to a constant weight at 105 °C. These extracts were prepared by homogenizing minced muscle with water [ratio 1:2 (w/w)] by means of an Omni Mixer 17220 (Sorvall) set at position 7 during two periods of 30 s each. Homogenates were then centrifuged at 15000*g* (15 min, 3 °C), and the supernate was collected quantitatively. The precipitate was subjected to two further washing steps subsequently, and supernates of each washing were mixed together. An aliquot of this mixture was dried to a constant weight at 105 °C.

Dimethylamine−Nitrogen (DMA-N) Analysis. Formaldehyde production was monitored by determining the content of dimethylamine−nitrogen according to the procedure of Dyer and Mounsey (1945) after extraction with trichloroacetic acid (Castell and Smith, 1973). FA production was expressed as micromoles of FA per 100 g of fish muscle, as FA and dimethylamine are produced in equimolar amounts via enzymic trimethylamine oxide demethylation. Results are the average of triplicate samples.

Thermal Analysis. Samples of each batch were subjected to differential scanning calorimetry (DSC) at subambient temperatures by using a DSC Pyris 1 (Perkin-Elmer) equipped with an Intracooler IIP mechanical accessory, which allowed measurements to be carried out down to −55 °C, and monitorized by the software Pyris (Perkin-Elmer) for Windows (Microsoft Corp.). Aqueous solutions of each maltodextrin and of sucrose [25% (w/w)] were also subjected to DSC analysis. Nitrogen (purity = 99.999%, water < 0.5 vpm) was used as purge gas. Heat flow and melting point were calibrated from the melting endotherm of indium (purity degree = 99.99%) and the melting temperature of water. The heating rate used for calibration was the same as that used for scanning samples to be analyzed. An empty pan was always used as a reference.

Samples (11−15 mg) were accurately weighed into 50 μL aluminum pans using a high-precision balance (± 0.0001 g) and immediately sealed with a Universal press (Perkin-Elmer). Once inside the calorimetric cell, pans were allowed to stand for 1 min at 10 °C and then cooled to −55 °C at a rate of 20 °C/min, being maintained at this temperature for 3 min. Afterward, samples were heated to 10 °C at 5 °C/min, and this latter thermogram was recorded for subsequent analyses. Scans were carried out in triplicate.

The ice-melting onset was defined as the onset temperature of the transition just preceding the ice-melting endotherm, its value being determined from the intersection of the tangents to the baseline and to the leading side of the transition. The percentage variation of the ice-melting endotherm area was obtained by means of the Pyris software by setting as integration limits a temperature slightly lower than T_m' and another one slightly above the ice-melting endotherm.

RESULTS AND DISCUSSION

As shown in Figure 1, DSC thermograms showed only one transition, which partially overlapped the ice-melting endotherm. Although theoretically T_g' defines the onset of ice melting, glass transitions usually occur over a temperature range, so T_g and ice-melting onset may be different (Roos and Karel, 1991a; Simatos and Blond, 1991, 1993). Consequently, discrepancies abound concerning whether the glass transition of fish muscle coincides with the onset of ice melting or occurs at lower temperatures (Nesvadba, 1993; Reid et al., 1995; Inoue and Ishikawa, 1997; Brake and Fennema, 1999). It was not the aim of this study to resolve this controversy. Accordingly, the concept of ice-melting onset has been used throughout the study, which does not mean to reject what might also represent T_g'. In fact, for maltodextrin−water binary systems, it seems that glass transition and ice-melting onset practically coincide (Levine and Slade, 1988, 1990; Ablett et al., 1993). In any case, this temperature defines the beginning of the range technologically important for the stability of frozen foods (Goff, 1995).

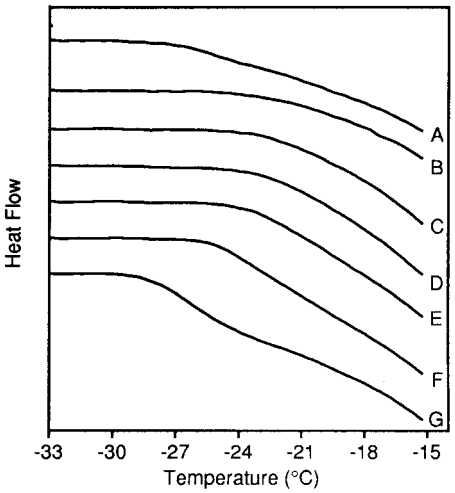

Figure 1. DSC thermograms of minced blue whiting muscle containing various cryostabilizers [8% (w/w)]: control (A); MD DE 4 (B); MD DE 9 (C); MD DE 12 (D); MD DE 18 (E); MD DE 28 (F); sucrose (G). Scans for one of the replicates are shown (replicate scans were similar to each other).

Table 1. Values of T_m'[a] (°C) for Minced Blue Whiting Muscle Containing Various Cryostabilizers [8% (w/w)] and for Their Respective Aqueous Solutions [25% (w/w)]

sample	minced fish	aqueous solution
control	−27.50	
MD DE 4	−23.00	−6.50
MD DE 9	−23.25	−9.75
MD DE 12	−23.50	−12.25
MD DE 18	−24.00	−15.50
MD DE 28	−25.50	−20.50
sucrose	−29.00	−34.50

[a] Measurements were carried out in triplicate (variation ± 0.5 °C).

The absence of a devitrification exotherm in those scans and the fact that previous studies showed that annealing had no effect on the ice-melting onset in these samples (Herrera et al., 2000) allowed acceptance of the freeze−concentration as maximum. Accordingly, the ice-melting onset, by analogy with T_g', was named T_m' (Roos and Karel, 1991a).

The addition of the different maltrodextrins assayed to minced blue whiting muscle increased T_m' by between 2 and 4 °C in comparison with the untreated control (Table 1). As discussed in Herrera et al. (2000), this increase was found to diminish with increasing DE, but not linearly. Thus, whereas the samples containing DE 4, 9, 12, or 18 maltodextrins showed T_m' values that

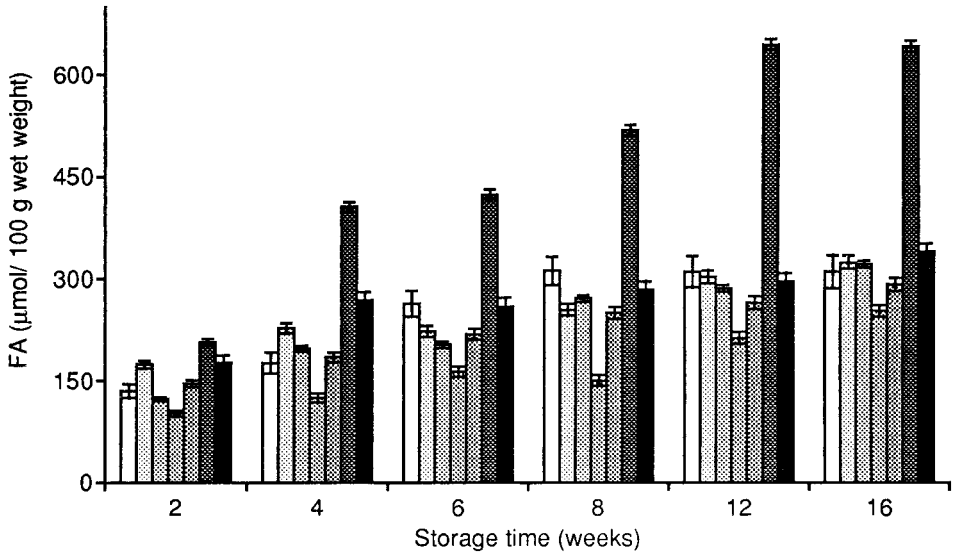

Figure 2. Formaldehyde production in minced blue whiting muscle containing various cryostabilizers during frozen storage at −20 °C. Bars represent MD DE 4, MD DE 9, MD DE 12, MD DE 18, MD DE 28, control, and sucrose from left to right at each storage period.

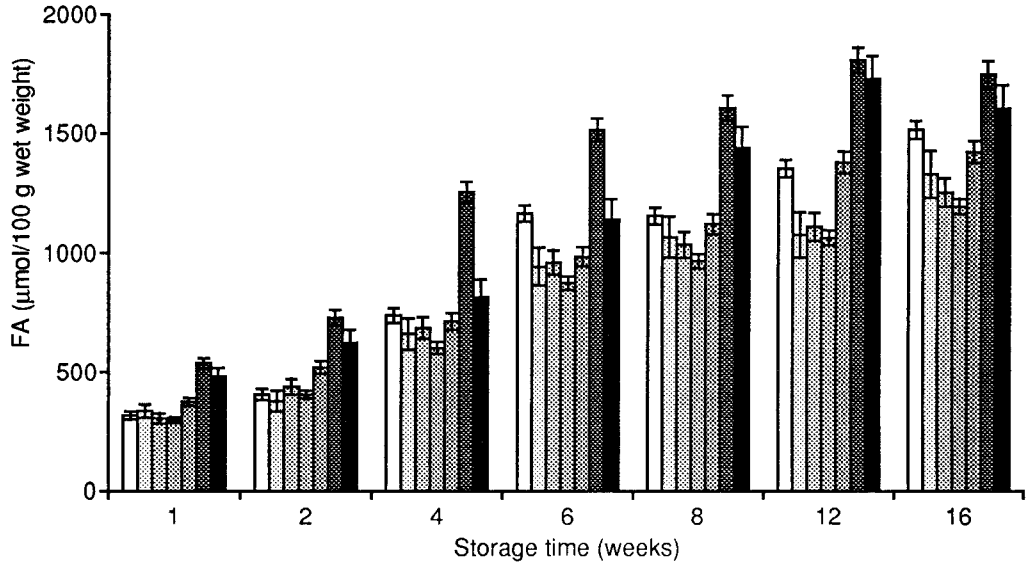

Figure 3. Formaldehyde production in minced blue whiting muscle containing various cryostabilizers during frozen storage at −10 °C. Bars represent MD DE 4, MD DE 9, MD DE 12, MD DE 18, MD DE 28, control, and sucrose from left to right at each storage period.

were very close together, that is, between −23 and −24 °C, that for the sample with DE 28 maltodextrin was somewhat lower. On the contrary, the addition of sucrose lowered T_m', which had a value of −29 °C. Thus, none of the samples was in the glassy state at the storage temperatures used in the present study.

The content of FA produced in the different samples during the storage at −20 °C is shown in descending T_m' order in Figure 2. At any sampling period, it can be observed that FA production decreased approximately with T_m' from the sample containing DE 4 maltodextrin to that with DE 18 maltodextrin, whereas it increased as T_m' decreased in the remaining cryostabilized samples. The highest values, however, were found in the control batch.

The production of FA defined a similar pattern during the storage at −10 °C (Figure 3) but, unlike at −20 °C, it was higher in the samples containing DE 28 maltodextrin than in those containing DE 9 or 12 maltodextrins. The samples with sucrose produced substantial amounts of FA, reaching levels close to that of the control.

It is therefore evident that these experimental results differ from those that WLF kinetics would predict if the universal values of the empirical coefficients C1 and C2 were accepted and either T_m' or T_g' was adopted as reference (Levine and Slade, 1988; Lim and Reid, 1991). According to this, the production rate would be expected to be proportional to the difference between the storage temperature and T_m'. In a similar sense, recent studies have shown that the WLF equation fails to correctly predict reaction rates in frozen systems when an experimental temperature, that is, a constant temperature, is taken as reference (Herrera and Roos, 2000).

To account for these discrepancies, it has to be borne in mind that the WLF equation was developed to describe the temperature dependence of some mechanical properties in constant-composition systems (Williams et al., 1955). Therefore, this equation could

define the kinetics of only diffusion-controlled processes in constant-composition systems. However, in frozen systems, the composition of the UFW phase, which is the system to be considered, changes with temperature as a consequence of ice melting. This has two opposite effects: a decrease in the viscosity, which would increase the reaction rate, and a dilution of the reactants, which would reduce it (Simatos et al., 1989; Simatos and Blond, 1991).

To take into account the variation in the composition of the UFW phase with temperature, Simatos and Blond (1991, 1993) proposed replacing T_g' with T_g in the WLF equation, the latter being the glass transition temperature corresponding to the composition of the UFW phase (C_g) at each storage temperature. Applying this criterion, Champion et al. (1997) were able to predict both the viscosity of the UFW phase and the enzymic hydrolysis rate of nitrophenyl phosphate in frozen sucrose solutions by means of the WLF equation provided that $T > 1.2T_g$.

State diagrams can be used to predict the variation of both T_g and C_g with temperature (Blond, 1989; Levine and Slade, 1990; Roos, 1995). Unfortunately, they can be characterized only for binary systems; it has not been feasible yet to extend their use to chemically complex systems. Faced with this limitation, recourse has been made to the study of simple model systems as an approach to that of more complex food systems (Karmas et al., 1992; Champion et al., 1997). However, in the present case, such an approach would not take into account the effect of fish muscle water-compatible solutes, so only a nonquantitative comparative study on the effect of the different cryostabilizers could be made.

Moreover, the fact that the temperature data used to build the *liquidus* curve are obtained under nonequilibrium conditions (Simatos and Blond, 1993; Pongsawatmanit and Miyawaki, 1993; Slade and Levine, 1995) becomes a major problem for concentrated solutions of high molecular weight polymers because kinetic restrictions prevent complete freezing (Roos and Karel, 1991b; Ablett et al., 1993). This would lead to a high degree of error at temperatures close to T_m', which are critical for the experimental conditions of the present study.

Being aware of the limitations associated with the characterization and use of the state diagrams for the maltodextrin−water systems and of the time needed for their construction, it was decided to use, alternatively, the percentage variation of the ice-melting endotherm area of the different samples (Figure 4) to compare the effects of the cryostabilizers on the freeze−concentration of fish muscle solutes in the UFW phase at both −10 and −20 °C. This alternative does not mean that it is possible to determine the variation of C_g, and consequently that of T_g, with temperature for each sample from the corresponding DSC thermogram. In fact, this would imply a notable error majorly due to an incorrect subtraction of the heat capacity background (Ablett et al., 1992a). Consequently, any dynamic correction that could eliminate the time lag in the response of the sample during the calorimetric analysis was omitted. Any reference to Figure 4 regarding the temperatures used in the present study will have to be taken only as approximate. This does not mean that the conclusions put forward will be any less valid.

On a percentage basis, blue whiting muscle contained ~5.5 g of water-soluble solutes and ~80 g of water. Accordingly, cryostabilized minced muscle, which consisted of 8 g of cryostabilizer (either sucrose or any maltodextrin) and 92 g of muscle, contained ~5 g of water-soluble fish muscle solutes and ~73.5 g of water (Figure 5). The remainder would be mostly proteins unable to be in the UFW phase.

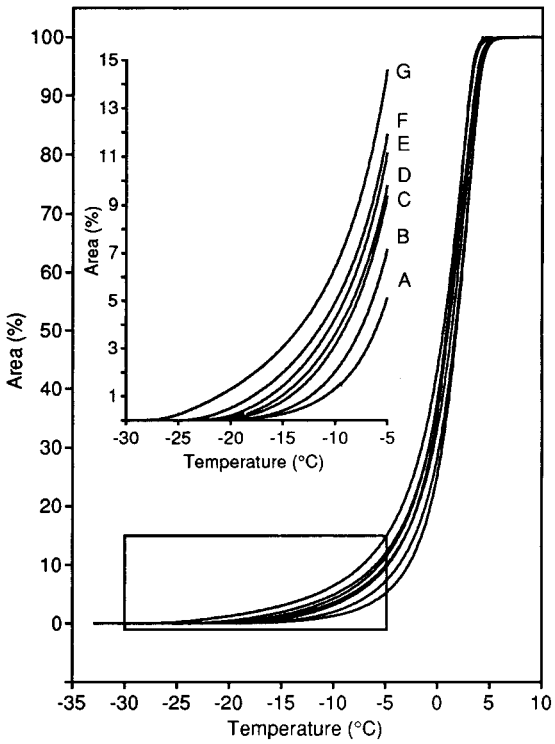

Figure 4. Percentage integral variation of the ice-melting endotherm area of minced blue whiting muscle containing various cryostabilizers. The curves are labeled as in Figure 1. Area (%) corresponds to the proportion of unfrozen water in relation to the total freezable water content. Variations for one of the replicates are shown (replicate scans were similar to each other).

CRYOSTABILIZED-MINCED FISH MUSCLE:

8 g CRYOSTABILIZER + 92 g MUSCLE (water: 80%)
⇓
5 g WATER-SOLUBLE SOLUTES + 73.5 g WATER + PHASE SEPARATED SOLUTES

COMPOSITION OF SAMPLE WITH SUCROSE AT Tm'

Assumption: Cg' (%) = 82

FROZEN WATER (96%) + PHASE SEPARATED SOLUTES + UNFROZEN WATER PHASE
⇓
8 g SUCROSE + 5 g WATER-SOLUBLE SOLUTES + ≈2.85 g UNFREEZABLE WATER

UNFROZEN WATER CONTENT IN SUCROSE-CONTAINING SAMPLE AT STORAGE TEMPERATURES

	-20°C	-10°C
Cg (%)	69.5*	57.5*
UNFROZEN WATER	7.8 (4.0)	13.1 (9.5)

*: data taken from sucrose-water state diagram (Blond et al., 1997).
Unfrozen water is expressed as g / 100 g water. Figures in parentheses are referred to the total freezable water content.

Figure 5. Approximate composition of the maximally freeze−concentrated UFW phase and rough estimates of unfrozen water content at −20 and −10 °C for the sucrose-containing minced blue whiting muscle.

In the case of the sample with sucrose, assuming that the effect of the water-soluble fish muscle solutes on C_g' (the content of solutes in the maximally freeze−concentrated UFW phase) is negligible, it can be assumed that the C_g' value for the sucrose−water system, that is, 82% (Hatley et al., 1991; Ablett et al., 1992b;

Blond et al., 1997) also applies to the UFW phase of this sample. The former assumption is justified by the fact that the C_g' values for different water−solute binary systems over a wide range of molecular weights are all ~80% (Hatley et al., 1991; Roos and Karel, 1991b; Ablett et al., 1992b; Roos, 1995). Considering that it is very unlikely that sucrose crystallizes, it becomes apparent by applying the C_g' concept that the composition of the UFW phase at T_m' would consist of sucrose (8 g), water-soluble fish muscle constituents (~5 g), and ~2.85 g of water "kinetically unfreezeable". Therefore, ~96% of the water of this sample would be frozen at T_m'.

If the same assumption is adopted for the values of C_g (the content of solutes in the UFW phase) obtained for the sucrose−water binary system at −10 and −20 °C [57.5 and 69.5%, respectively (Blond et al., 1997)], it follows that ~9.61 g (13.1%) and ~5.71 g (7.8%), respectively, of the water of the sample with sucrose would be unfrozen at those temperatures.

These calculations have been performed without taking into account the effect of the water-soluble fish muscle components on T_m'. Thus, it has been implicitly assumed that ice melting begins at a lower temperature than it does in fish muscle (see T_m' values for the aqueous solution of sucrose and the sample with sucrose in Table 1). Hence, the real C_g values for blue whiting muscle at those two temperatures would be higher than those obtained from the state diagram. To this effect, the variation of the ice-melting endotherm area for the sample containing sucrose (G) is consistent with this observation (Figure 4) and it shows proportions of unfrozen water somewhat smaller than those computed by ignoring the water "kinetically unfreezable", 9.5 and 4% water at −10 and −20 °C, respectively. It is important to remember that the water "kinetically unfreezable" is ignored in the ice-melting endotherm, so unfrozen water is referred to the total freezable water content.

The same calculations, unfortunately, could not be made for the aqueous solutions of the various maltodextrins assayed, as the characterization of the state diagrams of the maltodextrin−water systems would imply a substantial degree of error. However, assuming that C_g' has the same value for all batches, it is clear that the content of unfrozen water would be lower in the samples containing maltodextrins (B−F) than in that containing sucrose at both −10 and −20 °C (Figure 4). This can be explained in terms of the effect of the cryostabilizers on the colligative properties of the system, which depend on the number of particles.

In the case of the untreated control (A), the proportion of unfrozen water in relation to the total freezable water content was lower than in all of the cryostabilized samples at both storage temperatures (Figure 4). In keeping with these results, Gruda and Postolski (1986) showed values of 2 and 6% for such proportions in cod muscle at −20 and −10 °C, respectively, which were only slightly higher than those found for the control in the present study. Moreover, the addition of cryostabilizers not only increased the proportion of unfrozen water in relation to the total freezable water content but also had an additional effect by "diluting" the freeze−concentration of muscle components in the UFW phase. This could also be part of the reason for the lower production of FA in these samples.

This "diluting" effect is especially evident at temperatures around −20 °C, at which nearly all freezable water would be frozen (see Figure 4 and Table 2). Thus, small differences in the ice-melting endotherms would lead to significant variations in the freeze−concentration of fish solutes in the UFW phase and hence to significant differences in the reaction rates. In this respect, and going back to the calculations made from the sucrose−water state diagram, the content of water-soluble muscle components in the UFW phase of the sample with sucrose would be ~1.75 g/g of water at T_m', whereas contents of 1.41, 1.17, and 0.88 g/g of water would be obtained for proportions of unfrozen water in relation to the freezable water content of 1, 2, and 4%, respectively. On the other hand, in the case of the control, assuming that $C_g' = 82\%$, the content of muscle components in the UFW phase at T_m' would be ~4.55 g/g of water. This content would decrease to 2.75 g/g of water for a proportion of unfrozen water in relation to the freezable water content of 1% and to 1.98 and 1.26 g/g of water for proportions of 2 and 4%, respectively.

Table 2. Freeze−Concentration of Fish Muscle Solutes in the Unfrozen Water Phase (Grams per Gram of Water) of Control and Sucrose-Containing Samples

	unfrozen water[a] (%)						
	0	1	2	4	6	8	10
control	4.55	2.75	1.98	1.26	0.92	0.73	0.60
sucrose	1.75	1.41	1.17	0.88	0.70	0.59	0.50

[a] Unfrozen water values are referred to the total freezable water content. Values between 1 and 5% are to be found at −20 °C, whereas values between 5 and 10% are to be expected at −10 °C (see text for further details).

As can be easily inferred from Figure 4, the concentrations of muscle components in the UFW phase of maltodextrin-containing samples would be between those of the control and those of the sample with sucrose. Consequently, a higher T_m' and a lower freeze−concentration of reactants would explain the notable inhibitive effect of all of the maltodextrins at −20 °C. On the contrary, T_m' was slightly lower in the sample containing sucrose than in the control, but sucrose markedly inhibited FA production at this temperature. The reason for this seems to lie in a much lower freeze−concentration of solutes in the UFW phase (Table 2). This would also account for the differences found between the sample with sucrose and those with maltodextrins at this temperature, much smaller than those that would be predicted by the WLF equation if T_m' or T_g' was taken as reference.

Similarly, both T_m' and freeze−concentration of solutes in the UFW phase have to be considered when the different maltodextrins are compared. Thus, in accordance with the values for T_m', specific diffusion would be expected to be similar in all of the samples, perhaps proportional to the difference between the storage temperature and T_m'. On the other hand, assuming that C_g' is the same for all of them, the amount of unfrozen water would be proportional to the number of dextrose equivalents of the maltodextrin. Therefore, the lower the DE, the higher the freeze−concentration of solutes in the UFW phase, and so would be expected the reaction rate.

The result of these two opposing effects appeared as a slightly higher production of FA in the samples containing DE 4, 9, and 12 maltodextrins than in those containing DE 18 or 28 maltodextrins during storage at −20 °C, with the second effect being prevalent. However, the effect of T_m' would have prevailed between

these two latter samples, in which freeze−concentration would have been similar, and so DE 18 maltodextrin was more effective.

The content of unfrozen water was considerably higher at −10 °C than at −20 °C, with values in relation to the freezable water content ranging between 5 and 10%, depending on the sample (Figure 4). Therefore, the concentrations of muscle components in the UFW phase of the cryostabilized samples were closer to that of the control at −10 °C than at −20 °C (Table 2). This effect along with a lower restriction of cryostabilizers to molecular mobility would explain their lower effectiveness in inhibiting FA production at −10 °C. This was especially noticeable for sucrose, which had only a weak inhibiting effect at this temperature, as well as, although to a lower extent, for DE 28 maltodextrin, which, in accordance with their respective T_m' values, were much less effective than DE 9, 12, and 18 maltodextrins.

It has been previously pointed out that the profiles defined by the contents of FA produced in the different samples, when placed in descending T_m' order, showed a certain parallelism at −10 and −20 °C (Figures 2 and 3). Likewise, the percentage variations of the ice-melting endotherm areas of these samples were also parallel between these two temperatures (Figure 4). The differences in the freeze−concentration of solutes in the UFW phase seem to have determined, therefore, the differences in the production of FA among the samples containing DE 4, 9, 12, and 18 maltodextrins. Nevertheless, the low water solubility of DE 4 maltodextrin made it difficult for it to be homogeneously distributed in muscle. This could have also favored FA production, which reached levels similar to those of the control.

CONCLUSIONS

Methodological limitations avoid the variations of T_g and C_g with temperature to be predicted in complex food systems at present. Therefore, the cryostabilization of minced blue whiting muscle requires, on the one hand, that T_m' is not too low so as not to impose a notable restriction on formaldehyde production at the usual storage temperatures and, on the other hand, that freeze−concentration is not too high at relatively high storage temperatures because it could increase the rate of the process significantly. Thus, the choice of the cryostabilizer to be added must be made with the knowledge of the range of temperatures that the system may be subjected to. In any case it would be highly important to maintain, as far as possible, a constant temperature, or at the very least prevent it from reaching relatively high values that would promote the diffusion of reactants. However, temperature is not continuously controlled throughout the distribution chain of frozen foods but instead fluctuates, sometimes significantly.

Consequently, the results of the present study point to the need to consider replacing sucrose with other additives that, as may occur with maltodextrins, impose a greater restriction on molecular diffusion at relatively high frozen storage temperatures, thereby cushioning the effect of temperature fluctuations. Likewise, sorbitol, which is increasingly being used as a cryoprotectant in fish minces, would be expected to present similar or even greater problems because both its T_g' and T_m' are even lower than those of sucrose (Levine and Slade, 1988, 1990). This would in principle mean carrying out a further series of experiments on protein functionality, texture, and sensorial assessment as a basis for analyzing the pros and cons of such a substitution. In this sense, it is important to remark that several maltodextrins (DE 20−25) were found to be highly effective in preserving functional and sensorial properties of surimi of horse mackerel, a non-formaldehyde-producing species, during storage at −18 °C (Dondero et al., 1996). These authors also suggested that maltodextrins could be used as alternatives of sucrose and sorbitol during frozen storage of fish products and argued that they additionally reduced sweetness and had a similar or even lower cost.

Finally, it should be pointed out that considering how the rate of FA production changes during frozen storage (Herrera et al., 1999), the combined application of cryostabilization and a method that decreases the initial concentration of reactants, such as washing of muscle (Landolt and Hultin, 1982) or any of the protocols that enhance cofactor oxidation (Kelleher et al., 1981; Reece, 1985), would bring about a major reduction in their concentration in the UFW phase. It would be thus expected that such a combination increases the inhibiting effect of the cryostabilizer on the production of FA.

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