Ethylcellulose-Stabilized Heat Resistant Chocolate

by

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The ethylcellulose solvent substitution method was developed which added ethylcellulose solubilized in ethanol to molten chocolate and, after evaporation of the ethanol, produced heat resistance in the chocolate. Chocolate containing 2.17% ethylcellulose 10 cP had hardness of 18 N at 40°C measured by large deformation mechanical testing. The hardness of the chocolate was found to be dependent on the chocolate formulation and concentration of ethylcellulose, and independent of ethylcellulose viscosity. Mechanical testing on model systems revealed that polymer gelation of cocoa butter only played a minor role in the heat resistance observed. Instead, interactions between sucrose and ethylcellulose were responsible for the formation of a network within the chocolate that provided the majority of the mechanical strength and oil trapping at elevated temperatures. Atomic scale molecular dynamics simulations predicted the ability of ethylcellulose to hydrogen bond with sucrose and this was corroborated by Fourier – transform infrared (FTIR) spectroscopy. Scanning electron microscopy and mechanical testing showed the presence of an ethylcellulose -sucrose network that was able to resist deformation. Simulations predicted, and mechanical testing and FTIR, showed that lecithin, typically found at the surface of sucrose in chocolate, reduced heat resistance by impeding ethylcellulose -sucrose interactions. However, fluorescence microscopy revealed that the ethanol used to prepare the
chocolate could remove some of the lecithin from the sucrose. Furthermore, ethanol dissolved a small amount of the sucrose and both of these effects positively impact heat resistance. Finally, a solvent-free method of introducing ethylcellulose to food systems was explored by the development of thixotropic ethylcellulose oleogels. It was found that thixotropy could be achieved by matching the Hansen hydrogen bonding solubility parameter of the oil phase to that of ethylcellulose. This was demonstrated with an oleogel made with 8% ethylcellulose 10 cP and vegetable oil and glycerol monooleate at a ratio of 55:45. These two methods represent novel strategies to introduce ethylcellulose to food systems and the solvent substitution method demonstrated how ethylcellulose can be used to provide structure in foods.
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# TABLE OF CONTENTS

## CHAPTER 1
### GENERAL INTRODUCTION 1
1.1 Introduction 1
1.2 Objectives 4
1.3 Hypotheses 5
References 5

## CHAPTER 2
### HEAT RESISTANT CHOCOLATE 8
Abstract 8
2.1 Introduction 8
2.2 Strategies for inducing heat resistance in chocolate 11
   2.2.1 Enhancing network microstructure 12
      2.2.1.1 Development of a secondary a sugar network 12
      Direct incorporation of water 12
      Indirect incorporation of water 16
      2.2.1.2 Development of a stable, jammed particle network 26
      2.2.1.3 Development of a secondary network of high melting point emulsifier 27
   2.2.2 Addition of an oil/fat binding polymer 28
   2.2.3 Increasing the melting point of the fat phase 32
      2.2.3.1 Interesterification of the fat phase 32
      2.2.3.2 Addition of a high melting point fat 34
2.3 Conclusions 36
References 37
2.4 Additional Literature 42
References 44

## CHAPTER 3
### ETHYLCELLULOSE SOLVENT SUBSTITUTION METHOD OF PREPARING HEAT RESISTANT CHOCOLATE 45
Abstract 45
3.1 Introduction 45
3.2 Materials and Methods 47
   3.2.1 Materials 47
   3.2.2 HRC Preparation 48
   3.2.3 Heat Resistance Measurements 49
   3.2.4 Effect of EC Viscosity on Heat Resistance 49
   3.2.5 Measuring Ethanol Loss 49
   3.2.6 Effect of Thickness on Ethanol Loss 50
   3.2.7 Measuring Surface Lightness 50
   3.2.8 Oil migration 51
   3.2.9 Melting profile 51
### 3.2.10 Efficacy of other solvents for manufacture of HRC

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.10 Efficacy of other solvents for manufacture of HRC</td>
<td>52</td>
</tr>
</tbody>
</table>

### 3.2.11 Statistical analyses

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.11 Statistical analyses</td>
<td>52</td>
</tr>
</tbody>
</table>

### 3.3 Results and Discussion

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.1 Heat Resistance</td>
<td>52</td>
</tr>
<tr>
<td>3.3.2 Effect of EC viscosity on heat resistance</td>
<td>54</td>
</tr>
<tr>
<td>3.3.3 Drying of HRC</td>
<td>55</td>
</tr>
<tr>
<td>3.3.4 Effect of chocolate thickness on ethanol loss</td>
<td>57</td>
</tr>
<tr>
<td>3.3.5 Effect of drying treatment on surface lightness</td>
<td>57</td>
</tr>
<tr>
<td>3.3.6 Oil migration</td>
<td>58</td>
</tr>
<tr>
<td>3.3.7 Melting profile</td>
<td>59</td>
</tr>
<tr>
<td>3.3.8 Efficacy of other solvents for manufacture of HRC</td>
<td>60</td>
</tr>
</tbody>
</table>

### 3.4 Conclusions

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4 Conclusions</td>
<td>61</td>
</tr>
</tbody>
</table>

### References

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>62</td>
</tr>
</tbody>
</table>

### CHAPTER 4

#### MOLECULAR INTERACTIONS OF SUCROSE AND ETHYCELLULOSE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>64</td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>64</td>
</tr>
<tr>
<td>4.2 Materials and methods</td>
<td>67</td>
</tr>
<tr>
<td>4.2.1 Atomic scale molecular dynamics (MD) simulations</td>
<td>67</td>
</tr>
<tr>
<td>4.2.2 Hardness tests of model systems</td>
<td>70</td>
</tr>
<tr>
<td>4.2.2.1 EC interacting with sucrose</td>
<td>70</td>
</tr>
<tr>
<td>4.2.2.2 EC interaction with various particles</td>
<td>72</td>
</tr>
<tr>
<td>4.2.3 Fourier transform infrared spectroscopy (FTIR)</td>
<td>74</td>
</tr>
<tr>
<td>4.2.4 Scanning electron microscopy (SEM)</td>
<td>76</td>
</tr>
<tr>
<td>4.3 Results and discussion</td>
<td>77</td>
</tr>
<tr>
<td>4.3.1 Atomic scale MD simulations</td>
<td>77</td>
</tr>
<tr>
<td>4.3.2 Hardness tests of model systems</td>
<td>80</td>
</tr>
<tr>
<td>4.3.2.1 EC interacting with sucrose</td>
<td>80</td>
</tr>
<tr>
<td>4.3.2.2 EC interaction with various particles</td>
<td>83</td>
</tr>
<tr>
<td>4.3.3 Fourier transform infrared spectroscopy</td>
<td>87</td>
</tr>
<tr>
<td>4.3.4 Scanning electron microscopy</td>
<td>89</td>
</tr>
<tr>
<td>4.4 Conclusions</td>
<td>92</td>
</tr>
</tbody>
</table>

### References

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>92</td>
</tr>
</tbody>
</table>

### CHAPTER 5

#### THE ROLE OF LECITHIN AND SOLVENT ADDITION IN ETHYLCELLULOSE-STABILIZED HEAT RESISTANT CHOCOLATE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>96</td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>96</td>
</tr>
<tr>
<td>5.2 Materials and methods</td>
<td>98</td>
</tr>
<tr>
<td>5.2.1 Hardness tests of model systems</td>
<td>98</td>
</tr>
<tr>
<td>5.2.2 Scanning electron microscopy (SEM)</td>
<td>99</td>
</tr>
</tbody>
</table>
5.2.3 Karl Fischer titration 100
5.2.4 Fourier transform infrared (FTIR) spectroscopy 100
5.2.5 Fluorescence microscopy 101
5.2.6 Residual EtOH 102

5.3 Results and discussion 104
5.3.1 Hardness tests of model systems 104
5.3.2 Karl Fischer titration 108
5.3.3 Scanning electron microscopy 108
5.3.4 Fourier transform infrared spectroscopy 109
5.3.5 Fluorescence microscopy 111
5.3.6 Residual EtOH 116

5.4 Conclusions 117
References 117

CHAPTER 6
ENGINEERING THE THIXOTROPY OF ETHYLCYLLOLULOSE
OLEOGELS BY MODULATION OF POLYMER SOLUBILITY IN
TRIGLYCERIDE OILS 119

Abstract 119

6.1 Introduction 119
6.2 Materials and Methods 122
  6.2.1 Gel preparation 122
  6.2.2 Viscosity measurement 122
  6.2.3 Oil density measurement 123
  6.2.4 Hansen solubility parameter calculations 123
  6.2.5 Water vapour transmission rate 124
6.3 Results and Discussion 125
  6.3.1 Effect of GMO concentration 125
  6.3.2 Effect of EC concentration 128
  6.3.3 Effect of EC molecular weight 129
  6.3.4 Effect of surfactant type 130
  6.3.5 Effect of oil type 131
  6.3.6 Comparison to a commercial cosmetic product 133
    6.3.6.1 Water vapour transmission rate 133
    6.3.6.2 Viscosity 134
6.4 Conclusions 134
References 135

OVERALL CONCLUSIONS AND FUTURE WORK 137
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table Number</th>
<th>Table Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Types of chocolate used to manufacture HRC</td>
<td>47</td>
</tr>
<tr>
<td>3.2</td>
<td>Approximate drying times for HRC</td>
<td>56</td>
</tr>
<tr>
<td>4.1</td>
<td>Proportions of monomers in the EC30 molecule</td>
<td>69</td>
</tr>
<tr>
<td>4.2</td>
<td>Proportions of monomers in the co-block and ordered EC62 polymers</td>
<td>70</td>
</tr>
<tr>
<td>4.3</td>
<td>Particle densities and average diameters with standard deviations</td>
<td>85</td>
</tr>
<tr>
<td>4.4</td>
<td>Peak position (PP) and average peak shifts (ΔPP) with respect to the EC-free control observed in solvent substitution and heat method samples of sucrose with EC. The error is the standard deviation of three replicates</td>
<td>89</td>
</tr>
<tr>
<td>5.1</td>
<td>Formulas of samples used in the hardness tests</td>
<td>100</td>
</tr>
<tr>
<td>5.2</td>
<td>Peak position (PP) and average peak shifts (ΔPP) with respect to the EC-free control observed in solvent substitution samples containing phosphatidylcholine (PC) coated sucrose made with EtOH or ethyl acetate. The error is the standard deviation of three replicates</td>
<td>110</td>
</tr>
<tr>
<td>5.3</td>
<td>Concentration of residual EtOH in chocolate samples measured by enzyme assay</td>
<td>116</td>
</tr>
<tr>
<td>6.1</td>
<td>Ingredients used and their abbreviations and sources</td>
<td>122</td>
</tr>
<tr>
<td>6.2</td>
<td>Literature and calculated Hansen solubility parameters (in MPa^{0.5}) for gel components and combinations thereof</td>
<td>128</td>
</tr>
<tr>
<td>6.3</td>
<td>Hansen solubility parameters for SMO and its mixture of 40% in oil</td>
<td>131</td>
</tr>
<tr>
<td>6.4</td>
<td>Water vapour transmission rate (WVTR) of gel samples and a comparable commercial product*</td>
<td>134</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1.1 Ethylcellulose structure 3

Figure 2.1 Photograph and full spectrum autofluorescence micrograph of chocolate confection showing (A) cream filling, (B) milk chocolate coating, (C) white chocolate coating 11

Figure 2.2 Heat resistance of chocolate measured by a penetration test with needle probe. Adapted from Giddey and Dove, 1984 24

Figure 2.3 Melting points of chocolate with addition of cornstarch or gelatin. Adapted from Ogunwolu and Jayeola, 2006 29

Figure 2.4 Heat resistance of chocolate measured after 2 h at specified temperature with large deformation machine and cylindrical probe. Compound milk chocolate was used as a control; HRC contained compound milk chocolate with 2.22% EC cP 45 incorporated from a mix of 20% EC in anhydrous ethanol 31

Figure 2.5 Addition of higher melting fats to improve the heat resistance of chocolate. (A) Slip melting points of chocolate with cocoa butter (CB) replaced with interesterified CB (adapted from Bruse, Wallecan and Arruda, 2008). (B) Melting profiles of CB, mahua fat, and kokum fat and various combinations thereof (adapted from Jeyarani and Reddy, 1999). (C) Hardness of chocolate with CB replaced with kokum fat (tested at 30°C) (adapted from Maheshwari and Reddy, 2005) 33

Figure 3.1 Hardness of control (no EC) and solvent substitution compound milk chocolate with 2.17% EC 20 cP from a 20% EC in EtOH mix tested using a mechanical tester 53

Figure 3.2 Hardness of samples made with chocolate and EC 22 cP at 40°C. Numbers in brackets refer to concentration of EC in the EtOH mix 54

Figure 3.3 Hardness of samples at 40°C made with increasing amounts of EC of varying viscosity from a 20% EC in EtOH mix in compound milk chocolate. Different letters indicate statistically significant differences within that group (p<0.05) 55

Figure 3.4 Hardness at 40°C of compound milk chocolate made with increasing amounts of a mix of 20% EC in EtOH. Each point represents the averages and standard deviations of samples made with EC 4, 10, 20, and 45 cP 55

Figure 3.5 Hardness at 40°C of compound dark chocolate made with a 20% mix of EC of various viscosities in EtOH. Different letters indicate statistically significant differences (p<0.05) 56

Figure 3.6 Ethanol loss from HRC during storage 56

Figure 3.7 Rate constant K with standard error for EtOH loss from HRC of increasing thickness. The thickness is reported as the average and standard error of the thicknesses of samples at the three drying conditions. Drying condition refers to the temperature at which the EtOH was removed from the sample and "wrap" refers to samples wrapped in one layer of aluminum foil 57
Figure 3.8 Surface lightness of dried HRC. (a) Mold-contacting face; (b) atmosphere-contacting face. CMC is compound milk chocolate, HRC is heat resistant chocolate, and EtOH is ethanol control chocolate.

Figure 3.9 Oil loss after 10 days at 40°C from (A) HRC made with compound milk chocolate (CMC) and various concentrations of EC 20 cP and (B) control compound milk chocolates made with initial concentrations of EtOH equivalent to the initial concentration of EtOH in the samples with both EC and EtOH. EtOH was evaporated from all samples prior to oil migration testing.

Figure 3.10 Oil loss after 10 days at 40°C from HRC made with 2.17% EC of various viscosities in (A) compound milk chocolate (CMC) or (B) compound dark chocolate (CDC).

Figure 3.11 Comparing drying times for HRC made with EtOH or ethyl acetate (EA).

Figure 3.12 Heat resistance of (A) compound milk chocolate and (B) compound dark chocolate with EC 10 cP dissolved in EtOH or ethyl acetate (EA).

Figure 4.1 Structure of ethylcellulose with carbon and R-group numbering system.

Figure 4.2 Structure of (A) sucrose with carbon numbering system, (B) phosphatidylcholine, and (C) glucose.

Figure 4.3 Stick and space filling representations of: (A) POPC; (B) EC30 with hydroxyl groups indicated by dark spheres; (C) sucrose crystal. Images are not on the same scale.

Figure 4.4 Root mean square displacement (RMSD) of hydroxyl hydrogens on (A) ordered and (B) co-block EC polymers in triolein (simulation one).

Figure 4.5 Number of hydrogen bonds formed between: (A) ordered or (B) co-block EC and triolein (simulation one); and (C) ordered or (D) co-block EC and sucrose surrounded by triolein (simulation 2).

Figure 4.6 Average distances between EC donor and acceptors for: (A) ordered or (B) co-block EC in simulation three; and (C) ordered EC in simulation four.

Figure 4.7 Hardness of model chocolate samples at 40°C made using the (A) solvent substitution and (B) heat methods.

Figure 4.8 Hardness of model chocolate samples at 40°C made via the heat method with various (A) ratios of GMO:EC or (B) types of surfactant.

Figure 4.9 Light micrographs of various particles: (A) sucrose; (B) glucose; (C) native starch; (D) pre-gelatinized starch; (E) MCC pellets; (F) MCC fibers; (G) RBW; (H) glass beads; and (I) diamond. RBW and diamond are shown at a higher magnification and have a scale bar measuring 500 µm while all others have a scale bar of 200 µm.

Figure 4.10 Area under the displacement in compression curve at 40°C for samples made with various particles at increasing particle concentration and constant EC concentration. (A) sucrose;
(B) glucose; (C) native starch; (D) pre-gelatinized starch; (E) MCC pellets; (F) MCC fibers; (G) RBW; (H) glass beads; (I) diamond. Values represent the average and standard deviation. Black squares are the particles treated with EC in EtOH and grey circles are the control particles treated with only EtOH.

Figure 4.11 Photographs and SEM images of defatted chocolate samples at various magnifications. Columns show: (A) control; (B) EtOH control; and (C) 2.17% EC in chocolate. Sucrose is denoted as “s” and milk protein or cocoa powder is denoted by “p”.

Figure 5.1 Hardness of model chocolate samples made using the solvent substitution method with sucrose (suc), EC, lecithin (lec) and EtOH as the solvent.

Figure 5.2 Hardness of model chocolate samples made using the solvent substitution method with sucrose (suc), EC, lecithin (lec) and ethyl acetate as the solvent.

Figure 5.3 Hardness at 40°C of chocolates and ingredients treated with EtOH or ethyl acetate.

Figure 5.4 Hardness at 40°C of compound milk chocolate treated with EtOH.

Figure 5.5 SEM micrographs of defatted compound milk chocolate treated with increasing proportions of EtOH. Columns show increasing magnification of the same sample.

Figure 5.6 Micrographs of sucrose crystals coated in fluorescent phosphatidylcholine in oil. Micrographs A, C, and E were taken under bright field and B, D, and F were taken using fluorescence mode. Figures A and B show the control sample with no solvent; C and D show the sample treated with EtOH; and E and F show the sample treated with ethyl acetate. Arrows indicate areas where sucrose crystals appear to have fused.

Figure 5.7 Micrographs of sucrose crystals coated in fluorescent phosphatidylcholine in oil. Micrographs A, and C were taken under bright field and B, and D were taken using fluorescence mode. Figures A and B show the sample treated with EC in EtOH; and C and D show the sample treated with EC in ethyl acetate.

Figure 5.8 Micrographs of glass beads coated in fluorescent phosphatidylcholine in oil. Micrographs A, C, and E were taken under bright field and B, D, and F were taken using fluorescence mode. Figures A and B show the control sample with no solvent; C and D show the sample treated with EtOH; and E and F show the sample treated with ethyl acetate.

Figure 5.9 Fluorescence intensity at 534 nm of nitrobenzoxadiazole-labelled phosphatidylcholine in various solvents.

Figure 6.1 Appearance (A) and viscosity and shear stress with power law fit (B) of a sample made with 5% EC 10 cP GMO:HOSO 45:55.

Figure 6.2 Effect of surfactant concentration on viscosity of gels made with 5% (A) or 8% (B) EC 10 cP, HOSO and GMO.
Figure 6.3 Effect of EC concentration on viscosity of gels made with EC 10 cP and GMO:HOSO at 40:60

Figure 6.4 Effect of EC viscosity on the viscosity of gels made with 8% EC and GMO:HOSO at 45:55

Figure 6.5 Effect of surfactant type on viscosity and recovery of gels made with 8% EC 10 cP and a surfactant:HOSO ratio of 40:60

Figure 6.6 Effect of oil type on viscosity and recovery of gels made with 8% EC 10 cP and a GMO:oil ratio of 45:55

Figure 6.7 Effect of oil phase δₕ on the viscosity of gels made with 8% EC 10 cP and a GMO:oil ratio of 45:55
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
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<tr>
<td>ATR</td>
<td>attenuate total reflectance</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>CB</td>
<td>cocoa butter</td>
</tr>
<tr>
<td>DGDO</td>
<td>decaglycerol decaoleate</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimeter</td>
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<tr>
<td>EA</td>
<td>ethyl acetate</td>
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<tr>
<td>EC</td>
<td>ethylcellulose</td>
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<td>EtOH</td>
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<td>fraction 2</td>
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<td>HRC</td>
<td>heat resistant chocolate</td>
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<td>HSPs</td>
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<td>IE</td>
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<td>MCC</td>
<td>microcrystalline cellulose</td>
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<td>medium chain triglyceride</td>
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<td>MD</td>
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<td>NCVF</td>
<td>non-cocoa vegetable fat</td>
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<tr>
<td>O/W</td>
<td>oil-in-water</td>
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<tr>
<td>PC</td>
<td>phosphatidylcholine</td>
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<td>PKO</td>
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<td>POPC</td>
<td>1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine</td>
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<td>RBW</td>
<td>rice bran wax</td>
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<td>SMO</td>
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<td>water vapour transmission rate</td>
</tr>
<tr>
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</tr>
</tbody>
</table>
CHAPTER 1
General Introduction

1.1 Introduction

Heat resistant chocolate (HRC) is a term that researchers have used to describe chocolate which: does not melt; holds its shape; or resists deformation at temperatures above the normal melting temperature of chocolate which is around 34°C.¹ There is much interest in developing such a treat so that it can be sold during summertime or in tropical climates. Other instances where there has been a market for HRC include as a ration for military personnel to carry in their pocket, and for space travelers.² Consequently, for more than seventy years researchers have been working to find the perfect formula for a HRC. Despite all of this research no HRC has yet to be commercially successful.

This work aims to produce a heat resistant chocolate by gelling the fat phase of the chocolate using ethylcellulose (EC). A substance can be defined as a gel if it “has a continuous microscopic structure, with macroscopic dimensions that is permanent on the time scale of an analytical experiment, and it is solid-like in its rheological behaviour, despite being comprised mostly of liquid”.³ An organogel (or oleogel) can then be defined as a gel with oil as the liquid phase.⁴ EC organogels are polymeric physical gels meaning, that the gelling agent is a polymer and the network junctions are physical in nature (as opposed to chemical). Physical junctions can be formed via: entanglements; hydrogen bonding between side groups; ionic and ion-coordination bonding; or interaction of helices.⁵ Junctions are formed by groups on the polymer that preferentially interact with one another rather than with the solvent.⁵ Other organogelators exist many of which are low molecular weight molecules. However, very little information is available on other polymer gelators of non-aqueous solvents⁶ therefore, making the EC gels unique.

The first mention of EC as an organogelator came when it was used to gel ethoxylated glycerides.⁷ The use of a surfactant, namely Olivem, which is a mixture of cetearyl and sorbitan esters of fatty acids from olive oil, to modify the properties of EC organogels was first suggested by Ruiz Martinez et al.⁸ More recently our group under the supervision of Dr. Marangoni has been working on comprehensive studies of EC organogels including studies on the effect of
addition of a wide variety of surfactants as well as the role of the solvent phase composition among other things. EC organogels have been studied as a fat replacer; replacing fats high in saturated and trans-fatty acids with oils high in unsaturated fatty acids could offer heart health benefits. Since organogels retain some of the functional properties of fats such as their solidity they typically offer advantages over liquid oils by reducing oil migration and matching texture profiles to solid fats.

EC gels of vegetable oil are made by heating the EC and (optionally) surfactant in the oil with mixing until a clear solution is observed, which occurs just above the glass transition temperature of the EC, which is around 145°C. The gel sets upon cooling, the gelling temperature being dependent on the particular composition; with no surfactant present a gel of 9% EC 10 cP in canola oil sets at around 85°C. Observations have indicated that, when set, gels made with EC are sensitive to and lose some of their functionality as the result of shear. Unfortunately, chocolate should not be heated much above 45°C as there is risk of flavour changes and protein aggregation leading to an increase in viscosity. This temperature restriction makes it impossible to add an unset EC gel to conventional chocolate. Therefore, a low temperature method must be developed to incorporate EC into chocolate to gel the fat. To achieve this, the EC was first dissolved in ethanol (EtOH) then added to molten, conventional chocolate and finally the EtOH was evaporated from the chocolate. This will be referred to as the “solvent substitution” (SS) method. It was thought that using a volatile solvent such as EtOH would allow the EC to be incorporated into chocolate in its extended polymer state where it would be left to gel the fat in the chocolate when the EtOH evaporated. The heat resistance of various chocolates and the effect of chocolate type, EC concentration, and EC molecular weight will be tested using large deformation mechanical testing. The time to remove the EtOH from the chocolate will also be measured as this is an important parameter for manufacturers to know. Furthermore, other important characteristics of the chocolate will be studied including oil migration and melting profile.

Following the development of a heat resistant chocolate it would be important to study how heat resistance was achieved. This would help when trying to optimize the methods and formulations for production of the chocolate. A more thorough depiction of EC from a molecular level is required to further the understanding of EC-stabilized chocolate. EC is derived from cellulose.
and is a polymer composed of β-1,4 linked D-glucose units with some of the hydroxyl groups substituted with ethoxyl groups (Figure 1.1). Wood pulp or cotton linters are common sources of cellulose for preparation of EC. The cellulose must undergo a preliminary step that converts it to alkali cellulose by means of treatment with a strong solution of sodium hydroxide. The alkali cellulose is then ethoxylated by treatment with ethyl chloride. The EC is then washed and ready for use. Although EC is not yet widely used in food products it has generally regarded as safe (GRAS) approval in Europe and should soon have approval in the U.S. as well.

![Figure 1.1 Ethylcellulose structure](image)

As seen in Figure 1.1 there are three hydroxyl groups on each monomer (excluding terminal monomers) that are available for ethoxylation. The degree of substitution is controlled during manufacture and the EC polymers used in this study have a degree of substitution of 2.47-2.58 which allows for the EC to be soluble in organic solvents. Preliminary efforts to understand the mechanism of heat resistance in the chocolate will focus on determining if the EC is able to gel the fat phase and if there is a particular component of the chocolate that interacts with EC. From the literature review of HRC (Chapter 2) it became obvious that sugar played a big role in many of these previous formulations. Therefore, the possibility of an interaction between EC and sucrose will be explored. It is not obvious from the available literature if a chemical interaction exists between EC and sucrose. Considering the structure of both species it was thought that hydrogen bonding may occur between unsubstituted R groups on the EC chain and free hydroxyl groups on the sucrose crystal. In conventional chocolate, sucrose particles are often coated in a layer of emulsifier such as lecithin which helps the fat coat the sucrose allowing for better flow and optimal viscosity to be obtained. It is common to add around 0.5% soybean lecithin to
chocolate as an emulsifier\textsuperscript{18} though other emulsifiers such as mono- and diglycerides, or polyglycerol polyricinoleate can be used along or in combination with lecithin.\textsuperscript{19} Phosphotidylcholine (PC) and phosphatidylethanolamine (PE) both make up around 30\% of the phospholipids in soybean lecithin.\textsuperscript{13} PC shows good solubility in EtOH\textsuperscript{20} and PE is somewhat soluble in EtOH.\textsuperscript{21} Considering our system utilizes EtOH it is possible that the EtOH plays a role in the heat resistance by removing the soluble phospholipids from the surface of the sugar. The ability for EC from an ethanolic solution to interact with the various components of chocolate including the ability to gel the fat phase will be studied using mechanical testing of model systems. Atomic scale molecular dynamics simulations and Fourier-transform infrared spectroscopy will be used to study the specific interactions between EC and sucrose. Fluorescence microscopy will be used to determine if EtOH can wash away the lecithin from the surface of the sucrose. Finally, the structure of the chocolate will be examined by scanning electron microscopy.

Other potential methods to produce heat resistant chocolate by addition of EC will be explored. As mentioned above, the effect of surfactants on EC organogels has been studied and it was found that the addition of surfactant led to changes in the appearance and mechanical strength\textsuperscript{22} of the gels. These changes are thought to be due to a plasticization effect that the surfactant has on the EC.\textsuperscript{23} The addition of surfactants to EC oleogels will be studied for its ability to produce a thixotropic gel. Thixotropy is used to describe a material that shows shear thinning and will increase in viscosity when the shear is removed.\textsuperscript{24} It was thought that thixotropic properties would allow for EC gels to be incorporated into products at temperatures below the setting point of the gel without the shear present during manufacture causing a loss of functionality to the gel. A thixotropic gel could then potentially be used as a solvent-free alternative to the solvent substitution method to produce heat resistant chocolate. The thixotropy of EC oleogels made with various surfactants will be studied using rheology.

1.2 Objectives

The main objectives of this thesis are:

- To develop a method for incorporating EC into chocolate in such a way that heat resistance is achieved without compromising, to a reasonable extent, the quality of said chocolate
• To determine a possible mechanism responsible for providing heat resistance in solvent substitution chocolate
• To develop a thixotropic EC oleogel

1.3 Hypotheses

The following hypotheses will be addressed throughout the thesis:

• A heat resistant chocolate can be made by gelling the fat phase of the chocolate using EC.
• A heat resistant chocolate can be manufactured by adding EC dissolved in EtOH to a conventionally manufactured chocolate and thereafter evaporating the EtOH from the chocolate.
• In solvent substitution chocolate EC and sucrose are able to hydrogen bond to one another leading to structure formation that provides heat resistance in the chocolate.
• EtOH is able to remove some of the phospholipids, namely PC, from the sugar surface, allowing the surface of the sugar to be available for interaction with EC.
• A thixotropic EC oleogel could be developed by highly plasticizing the EC through addition of surfactants.

References


CHAPTER 2

Heat Resistant Chocolate

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Abstract

The development and production of heat resistant chocolate (HRC) would allow this delectable treat to be enjoyed in summertime and in tropical climates. Chocolate generally melts at 33.8°C when solid cocoa butter transitions to liquid (DeMan, 1999). It is desirable to increase this melting point, or structure chocolate in such a way that it remains solid-like at elevated temperatures. A review of known methods and formulas for the production of HRC was undertaken. Three main approaches to create HRC were found: enhancement of the microstructure of the materials, addition of a polymer and increasing the melting point of the fat phase. The network strength and thermal stability could be enhanced by the development of a sugar network, addition of a high melting emulsifier or the creation of a jammed particle network. Addition of a polysaccharide polymer and increasing the melting point of the fat phase were also common strategies found. Many strategies exist to generate a sugar network in chocolate including: incorporation of water by direct or indirect (such as by hygroscopic action of humectants) methods; and processing the chocolate in such a way that some surfaces of the sugar remain uncoated by fat. The main drawbacks of the methods of producing HRC by water addition were an increase in chocolate viscosity, a higher propensity to develop undesirable sugar bloom, the logistical problems associated with the removal of the water by evaporation after heat resistance was developed, and the associated increased costs of manufacturing. Addition of non-conventional ingredients (high melting fats, surfactants, polyols) on the other hand would be limited by the standards of identity of chocolate in most countries.

2.1 Introduction

Chocolate is a food product that is loved by many because of its desirable qualities. These qualities include a shiny gloss on the surface, snap when the chocolate is broken, and a smooth texture that becomes apparent only when the chocolate melts in the mouth. There is however a
problem with chocolate in summertime and in most warm tropical countries – chocolate melts. Cocoa butter, the main fat constituent in chocolate, melts at 33.8°C when cocoa butter crystals are in the stable form V (DeMan, 1999). A thorough search through the literature for solutions to this problem was undertaken. The processes and formulas to produce heat resistant chocolate (HRC) are outlined here and critiqued. Furthermore, similarities amongst the different strategies were examined. Finally, a review of the most promising solutions and recommendations for future work are given. This concise overview of past efforts to formulate HRC will outline known difficulties and successes that have previously been encountered.

Conventional chocolate manufacture consists of the multiple steps discussed below with some minor variations (Beckett, 2000; Hui, 2006, 2007). After harvesting, fermentation and sun drying, cocoa beans are cleaned via magnetic removal of metal contaminants and elimination of stones etc. via particle density separation. The beans are then subjected to roasting and thereafter winnowing which is the process that removes the shell from the nib of the cocoa bean. Impact plates with vibrating sieves are used to complete this process. The cocoa nibs are then ground using a variety of mills. The resulting chocolate liquor is then mixed with sugar, extra cocoa butter, milk solids ingredients, emulsifiers, and flavours. The final chocolate should contain around 30% fat, 20% cocoa powder and 50% sugar. If a white chocolate is to be produced, cocoa solids are replaced with whole milk powder or something similar which introduces milk fat and milk solids non-fat into the chocolate. The mixture proceeds to refining which is a process that reduces the particle size of the solids in the chocolate using steel rollers. Shear mixers called conches are then used in combination with temperature and moisture control to obtain the desired product. Conching can alter chocolate rheology, remove volatiles, remove moisture, and improve mouthfeel. Emulsifiers, flavours and more cocoa butter can be added to the product during this step. The chocolate must be standardized for viscosity. Lecithin is added as an emulsifier to lower the viscosity of the chocolate. A low plastic viscosity is desired. The chocolate is then tempered. Tempering is the controlled cooling of melted chocolate with agitation. This nucleates cocoa butter crystals in the proper polymorphic form which then templates the entire crystallization process throughout the mass. Specifically, the chocolate is heated to 43-46°C. At this temperature the fat crystals have melted. The chocolate is then cooled with agitation to 24-29°C and reheated to 30-31°C prior to subsequent processes. The tempering conditions vary depending on the cocoa beans used. Specifically, the location of bean growth can affect the
tempering procedure because the melting point of the cocoa butter varies slightly with the growing temperature. The amount of cocoa butter present will also factor into the tempering conditions required. The tempered chocolate can be moulded or used for enrobing. Chocolate is then slowly cooled initially to 18°C and then down to 7°C. Generally, chocolate is packaged by cold sealing with water-based, low odour adhesives. The packaging must be a barrier to oxygen, moisture, and light. The finished chocolate should be stored at 18-20°C and a relative humidity of less than 50% with little to no contact with light.

The structure of chocolate can be imaged using various microscopy techniques. Figure 2.1 shows a photograph of a commercial chocolate product which has been sectioned using a knife as well as an autofluorescence micrograph of the cross-section. One can discern the white and milk chocolate marbled coating and a chocolate cream filling. The microscopy technique used here is full spectrum autofluorescence following UV excitation imaged with a Leica DM RXA2 upright light microscope equipped with a CRI Microcolor II® red, green, blue, violet (RGBV) liquid crystal filter and a Retiga® 1300 monochrome camera. This image shows that chocolate can be described as a continuous network of fat with solid particles embedded throughout. The use of UV fluorescence allows for differentiation of the various particles found in chocolate. Yellowish, angular particles are lecithin-covered sugar crystals, while bluish round particles are cocoa powder. This technique also allows for the size of the solid particles in the chocolate to be estimated. The particles should be a maximum of 15-30 μm in size and achieving this is critical to the final texture of the chocolate.

The careful manufacture and storage of chocolate can produce a high quality product. However, two main quality defects which are associated with chocolate are sugar and fat bloom (Timms, 2003). Sugar bloom occurs when water contacts chocolate and dissolves the sugar in the chocolate. Upon evaporation of the water this dissolved sugar re-crystallizes on the surface of the chocolate in a thin film. The appearance of the chocolate is marred by the greyish coloured sugar crystals. Furthermore, sugar bloom is expressed as an unpleasant, rough, and grainy mouthfeel. Fat bloom on the other hand is a result of the growth of the solid fat phase. One of the most commonly accepted mechanisms responsible for fat bloom formation is the transformation of the desirable triclinic form V to an undesirable triclinic form VI polymorph in cocoa butter. This transformation is associated with the development of large crystals at the chocolate surface.
which scatter light and give a whitish appearance. Fat bloom has been studied extensively and several mechanisms have been proposed (Timms, 2003).

![Figure 2.1](image)

Figure 2.1 Photograph and full spectrum autofluorescence micrograph of chocolate confection showing (A) cream filling, (B) milk chocolate coating, (C) white chocolate coating

Another difficulty with chocolate manufacture is seizure. Seizure occurs when small amounts of water, or polyols, are incorporated into chocolate. The water dissolves some of the sugar molecules which then become sticky and bind to various particles in the chocolate preventing flow and resulting in an unusable solid-like chocolate mass (Figoni, 2007).

### 2.2 Strategies for inducing heat resistance in chocolate

The amount of articles in peer reviewed journals concerning HRC and the like was surprisingly low. However, numerous patents from around the world indicate that much research on this topic has been done. It is a lucrative subject area in which many people have invested both time and money in hopes of finding the magic formula. Literature reviewed revealed three main strategies to produce HRC: enhancing network microstructure, addition of an oil/fat binding polymer, and
increasing the melting point of the fat phase. All percentages reported below are on a weight/weight basis (except for relative humidities).

2.2.1 Enhancing network microstructure

2.2.1.1 Development of a secondary a sugar network

Direct incorporation of water. First introduced by Lataner (1949), it was suggested that HRC could be prepared by the addition of water to conventional chocolate. This method specified the addition of 4-20% water to chocolate and heating to >65.5°C such that most of the sugar in the chocolate was dissolved in the water. This product was mixed at the elevated temperature until homogeneous. The product was then cut, extruded, or pressed into a desired shape. Optionally, the solid product was then ground and subsequently converted to a syrup by addition of warm water. The syrup could be used for coating or enrobing confections. The chocolate produced was said to be able to withstand any climatic conditions found in any part of the world. The patent specifies that the chocolate should have 28-33% fat, 46-55% sugar with a ratio of fat to sugar that is greater than 5:7 and combined should not exceed 88% of the total product. The balance of the total must be a non-crystallizable powdered material such as cocoa powder and/or milk powder. Some of the added water was evaporated off during the heating and cooling processes. It was found to be advantageous to achieve a final water content of 4-10%. Although not directly mentioned it seems that this product has properties such as a high viscosity that make it difficult to mould. This high viscosity would be hard to work with and would limit the product’s usefulness.

Russell and Zenlea (1948) developed a similar method. Warm water (48.9-65.6°C) was incorporated at levels of 17-23% into molten refined chocolate paste (43.3-54.4°C) having a fat content of 23-28% The chocolate paste and water were stirred continuously in a steam jacketed mixer until a homogenous, flowing mix was produced. Dipping and/or enrobing of articles occurred as in normal processing when the mix reached a temperature of 32.2 – 71.1°C. Chocolates were then dried at room temperature, in the presence of a current of dry air at temperatures of 21.1 – 43.3°C, or under partial vacuum to evaporate the water from the chocolate. The heat resistance of this chocolate coating was such that it did not exhibit flow behaviour at temperatures of about 37.8 – 48.9°C.
One could speculate that the use of refined chocolate paste as opposed to conventional chocolate in this method would aid in dissolving the sugar in the chocolate. This is likely the case because sugar molecules in refined chocolate have not been fully coated with fat as this occurs during the subsequent conching step of processing. One concern with the use of refined chocolate paste for this formula would be that it did not have a “finished” flavour which is developed during the conching stage of processing (Beckett, 2000). Instead, undesirable astringent and acidic flavours are evident in refined chocolate paste and may therefore exist in the HRC produced by this method (Timms, 2003).

Noznick and Obenauf (1963) used technology similar to that described above to develop a chocolate chip which resisted softening and melting and therefore was advantageous for use in baking mixes where discrete chocolate chips are desired. In this invention 4 parts chocolate was added to 6 parts water at 63°C, homogenized to create an oil in water emulsion (O/W) and then spray dried. This part of the procedure is similar to that described by Zizinia and McKenna (1940) in their production of a powdered chocolate liquor. In this patent however, sugar is present in the formula. The resulting powder was then dispersed into melted chocolate at a ratio of about 6:10 powder to chocolate and mixed for 3-5 h at 29-31°C. It was found that the greater percentage of powder added, the greater the heat resistance achieved with 5:10 being the lowest ratio giving a substantially heat resistant product. A similar product was produced by mixing chocolate liquor with 60% water, heating, homogenizing, spray drying and subsequently mixing the unsweetened product with a sugar. This powder was then added to conventional chocolate as above. It was found that chocolate made in this manner did not objectionably soften at temperatures greater than 46°C; some formulas resisted softening up to 120°C. It is likely that chocolate produced in this manner would have a high viscosity. About 35% of the fat, that which is provided to the total by the powder, was encapsulated and therefore unable to coat the solid particles and enhance their free-flowing abilities.

In a method similar to that of Noznick and Obenauf (1963), Jeffery, Glynn and Khan (1977, 1978) were able to produce HRC by creating an O/W emulsion with chocolate and water and thereafter evaporating off the water. A mix of about 15% water in chocolate was prepared. Optionally, an emulsifier in permissible amounts could also be added to the mix. This mixture was then homogenized or mixed at high speeds creating an O/W emulsion. A vacuum evaporator
was then used to decrease the moisture content in the chocolate to approximately 10%.
Thereafter the chocolate was shaped/moulded and dried to a moisture content of less than 5%.
This method produced a chocolate that did not flow up to temperatures of 65.5°C. Jeffery et al. (1977) proposed that heat resistance was accomplished by an encapsulating cocoa butter into fat globules surrounded by a sugar glass layer thereby preventing seepage of oil when the fat melted at elevated temperatures (Jeffery et al. 1977). These researchers also proposed that the chocolate could be produced in expanded form by flash heating the reduced moisture paste under vacuum. The density of the product would be decreased and shorter drying times would be achieved. It was also suggested that the chocolate product could be enrobed in a chocolate coating to provide an initial conventional chocolate texture.

The heat resistance of the chocolate achieved via the aforementioned method was substantially increased from conventional chocolate, resisting physical breakdown up to temperatures of 65.5°C. Furthermore, the chocolate produced conformed to ingredient limitations set forth by many countries (Canada, 2009). However, to remove such a large amount of water from the product and to homogenize chocolate would require a substantial change in the way chocolate is manufactured, requiring additional equipment, such as a homogenizer and a vacuum evaporator. This could result in a prohibitive increase in the cost of manufacturing (Potter & Hotchkiss, 1998). The product in its expanded form would also be quite unlike conventional chocolate products in its described appearance and texture. Sensory evaluation needs to be completed to ensure consumer acceptability of this product. The mechanism by which heat resistance was attained needs to be further studied. A sugar glass coating around fat globules seems somewhat unlikely considering sugar is hydrophilic (Fennema, Damodaran, & Parkin, 2008) and would likely remain dissolved in the aqueous phase until the water is removed. Perhaps this leaves behind a sugar network which is not necessarily surrounding the fat phase but merely entrapping it providing a network which immobilizes liquid oil. Problems with sugar bloom may also be encountered due to the presence of water in the chocolate.

In the 1979 patent by Jeffery, HRC was developed by a similar process with the production of an O/W emulsion that contained a permitted gum and/or gelling agent (such as gelatin). The emulsion was added at levels that incorporated 1-2% gum or gelling agent into conventional chocolate. The resultant chocolate had a chewy texture.
A method of producing HRC was developed by Takemori, Tsurumi, Takagi and Ito (1993) which was a slight modification of those mentioned above. This method involved forming an O/W emulsion with 20-70% water, 30-80% fat, and 1-20% emulsifier and a droplet size of 0.1-20 µm. This emulsion was then added at levels of 1-10% to tempered conventional chocolate. This process was advantageous in that the chocolate did not rapidly increase in viscosity during incorporation of the emulsion and therefore was easily mouldable. The chocolate was able to maintain its shape at 50°C after 10 days of aging.

In their 1991 patent Giddey and Dove attempted to improve the distribution of water into chocolate by incorporating the water as a foam. The foam was prepared by adding 0.1-30% surfactant to water and whipping in the presence of a suitable gas (air, nitrogen, carbon dioxide etc.) to create homogenous foam with a density of 0.05-0.2 g/mL and bubbles with a diameter of 0.1-100 µm. Advantageously, viscosity enhancers such as polyols or sugars could replace 5-30% of the surfactant in the foam to enhance stabilization and decrease the total amount of surfactant needed. Similarly, 0.1-2% of a cross-linked polypeptide or carbohydrate such as pectin, xanthan, or carrageenan could be added to the foam for viscosity enhancement. The foam was stirred and kneaded with molten, tempered chocolate (29-31°C) for a few minutes. Enough foam was added to incorporate 0.1-5% water into the final chocolate. The chocolate was then moulded and solidified. Optionally, prior to solidification the chocolate could be degassed by subjecting it to reduced pressure or by mechanical pressing. It was also found that distribution of water could be improved by storing the chocolate at 27°C for 12-48 hr following solidification. The chocolate produced remained solid up to 50°C. This method required some non-conventional chocolate making equipment which could increase the manufacturing cost. Furthermore, stability of the foam was important and immediate use following production was preferred.

Some drawbacks to these inventions included that the drying or degassing processes may have removed desired flavour volatiles and represented an expensive and time consuming process (Potter & Hotchkiss, 1998). Furthermore, any chocolate system that includes water in the formula could be faced with the problems associated with sugar bloom and its effects on the quality of the chocolate.
Indirect incorporation of water

Incorporating a humectant. In his attempt to create a “soluble” chocolate that incorporates flavourings or medicinal ingredients, Friedman (1921) inadvertently created a chocolate that could stand temperatures up to 49°C. The sweetener used in this chocolate formula was a mixture of one-third corn syrup and two-thirds sucrose. This mixture was boiled under pressure to remove the water and then finely ground. This sweetener was then added to the other conventional chocolate ingredients and mixed for a relatively short time using a refining machine. The resulting chocolate was then moulded and solidified in the conventional manner.

This product was not subjected to the conching process and therefore the sugar particles would remain partially uncoated by the fat in the chocolate. It is likely that heat resistance in this product was achieved by the development of a sugar network that traps the liquefied fat when temperatures exceed the fat’s melting point. The interaction of the partially uncoated sugar particles allowed for the formation of such a network. Any moisture in the chocolate would aid in the formation of this sugar network. This moisture could be from the chocolate ingredients including the sweetener if the drying process failed to remove all moisture, or by hygroscopic action of the uncoated sugars in the chocolate.

This chocolate likely had a high viscosity, poor texture and an unfinished flavor due to the absent conching step. Furthermore, the processing of the sweetener requires special equipment and increases the time required to manufacture this chocolate. As previously mentioned, any water in this chocolate could lead to sugar bloom and a decrease in the quality of the chocolate.

Similarly, Kempf and Downey (1956) developed HRC by using a conventional chocolate formula and a shortened conching process to prevent solid particles, specifically sugar particles, from being completely coated with fat. The viscosity of the semi-conched molten mass was too high to allow for enrobing. Therefore, to reduce the viscosity 2-3% water was added to the chocolate. During the conching process, the water wetted the non-fat solids and further working led to an unstable coating of fat on the surface of these wetted particles. This gave temporary fluidity to the mass allowing for enrobing. The resulting chocolate resisted deformation however, it was subject to oiling off when the fat melted. To prevent oiling off, moisture could be applied to the finished chocolate product such that the surface sugar crystals were dissolved and upon
evaporation of the water, recrystallized to form a thin skin of sugar on the surface. The film of sugar thus acted as a barrier between the packaging material and the oil from the melted fat in the chocolate. Roberts (2003) used a similar sugar coating method to give heat resistance to a low-density (gas pocket containing) chocolate. The mechanism of heat resistance would be similar to that described above. The disadvantages of Friedman’s (1921) work described above also apply here.

Attempts were made by Kempf (1958) to make a product which could be used by a confectioner to prepare HRC with a “finished” chocolate flavour. This was accomplished by producing a chocolate in the conventional manner but which contained 0.66-6.6% milk protein (casein and lactalbumin), 42.5% sucrose coated with 0.85-2.13% moisture and 0.13-0.80% invertase. The completed chocolate was left to absorb 0.4-2.5% moisture which was necessary for the expression of its heat resistant abilities. The proposed mechanism by which this formula provided heat resistance was as follows: the water on the sucrose allowed for invertase enzyme to hydrolyze sucrose to inverted glucose and fructose. The inverted sugars are very hygroscopic thus when exposed to high humidity conditions water was absorbed. This water was transferred to the milk proteins which then swelled resulting in stronger binding between the proteins, creating a secondary network within the chocolate. This structure did not melt at elevated temperatures (above 33.8°C) and therefore provided a means of containing melted fat and providing mechanical rigidity which resulted in the observed heat resistance.

The main benefit of this invention was that a confectioner could receive the chocolate in its non-heat resistant form, work the chocolate into its desired shape then expose the chocolate to humidity to render it heat resistant. Another advantage of this method was that humectant action was retarded until after final shaping of the chocolate, the retardation being controlled by the amount of invertase and moisture coating the sugar. This was important to prevent seizure of the chocolate during processing. However, careful control of the humidity during processing would eliminate the need for postponing hygroscopic action but this would be an added manufacturing cost. Setbacks with the invention include the aforementioned problems with sugar bloom. Furthermore, the moisture absorbing process was reported to take 14-28 days at a relative humidity of 50-70% which is a relatively long time. Another drawback of this invention is the high cost of enzymes (Fennema, Damodaran, & Parkin, 2008). This patent lacks any evidence of
the heat resistant abilities of the invention. Furthermore, the structuring mechanism provided by the milk proteins is an interesting proposal which requires research to provide evidence for its existence. It is likely that the moisture in this chocolate allowed for sugar particles to bind to one another creating a sugar network. Perhaps it is both a sugar network and protein network that lead to heat resistance in this chocolate.

O’Rourke (1959) proposed a simplification of Kempf’s (1958) enzyme-mediated process of incorporating humectants into chocolate. The HRC could be made by adding a humectant and milk protein to a chocolate formula then exposing the finished chocolate to a humidity of 50-70% for 14-28 days. O’Rourke recognized that for heat resistance to be achieved the relative humidity in the facility must not be more than 45% during processing of this humectant containing chocolate, especially during refining. Humectants such as corn syrup solids, glucose syrup solids, dextrose, maltose, invert sugar, amorphous sucrose, glycerin, and sorbitol could be added to chocolate at any suitable level. It was found that increasing the amount of humectant in the chocolate led to increased moisture absorption resulting in an increase in heat resistance (up to a maximum).

This process was simpler than Kempf’s (1958) because it did not require sucrose to be coated with enzyme and moisture. However, some of the money saved by not using enzymes would be spent on maintaining low relative humidity in the processing facility. All other advantages and disadvantages mentioned for Kempf’s (1958) process also apply here. Furthermore, the effectiveness of this treatment was not reported.

Shubiger and Rostagno (1965) developed a method of producing HRC that combined non-conched chocolate made with amorphous sucrose with conventional chocolate. A development period of 10-60 days at 20-35°C was required for the sugar network and resulting heat resistance to form. The chocolate had a finished chocolate flavour due to the combination of non-conched and fully conched chocolate. However, the uncoated amorphous sugar likely contributed an unpleasant texture to this chocolate. Giddey and Menzi (1966) have described a similar process. Furthermore, Pirsch, Shubiger and Rostagno (1971) refined this method. This patent specified that the amorphous sugar used should have a particle size not exceeding 20 µm. It was found that the heat resistance achieved was directly related to the particle size of the amorphous sugar. In
these patents the uncoated amorphous sucrose likely formed a sugar network that trapped the liquid fat at elevated temperatures as described previously. The development period probably allowed for water to be added to the chocolate via hygroscopic action of the amorphous sucrose. As previously mentioned, the water aids in the formation of the sugar network.

Finkel (1987, 1989, 1990) suggested a HRC formula based on adding a liquid polyol at levels of 0.2-5.0% to chocolate. The polyol was incorporated into molten chocolate after the chocolate was tempered. The mixture was held or moulded and held at 28.9-32.8°C for a sufficient amount of time to allow the fat and polyol to interact. The holding time depended on the fat content, concentration of polyol, temperature, and viscosity requirements for the product. A particular formula of HRC prepared with 1.0% glycerin exhibited slight softening but was not fluid at temperatures of 48.9°C whereas a control chocolate without glycerin melted completely at this temperature. Researchers believed that when the liquid polyols were added to chocolate they chemically interacted with the fat in the chocolate. This interaction led to an increase in the viscosity of the mix. Furthermore, the viscosity of the mix was found to increase with increasing time and/or temperature as well as with increasing amounts of polyol up to a maximum. This interaction could be a part of the mechanism leading to HRC produced by the addition of polyols.

This formula and procedure was very simple and products displayed some resistance to heat. However, Davila and Finkel (2002) report that this method created a chocolate that set within seconds making it difficult to produce in a commercial setting. Special equipment which adds the polyol just before moulding or enrobing was suggested to avert this problem however this is potentially problematic and costly. Further research into the mechanism by which polyols improve the heat resistance of chocolate is needed.

The use of polyols to make HRC as suggested by Finkel (1987, 1989, 1990) can be considered as similar to the other methods described in this section. All of the formulas developed included a hygroscopic material and chocolate containing milk proteins (except the work by Friedman (1921) which did not specify addition of milk ingredients). Finkel (1987, 1989, 1990) did not express a need for milk proteins however, skim milk powder, which contains protein, was included in the example recipes. Furthermore, although Finkel did not describe a need for
moisture absorption, polyols are hygroscopic (Fennema, Damodaran, & Parkin, 2008) and polyols in the liquid state were necessary for HRC to be produced. Therefore, water may have been incorporated due to polyols absorbing water during storage, low purity ingredients, or absorption post-manufacture. Whether water was incorporated or not, HRC produced with polyols would likely be prone to sugar bloom and its deteriorative effects due to the hygroscopic nature of this additive.

Work by Kincs (1990) produced a HRC similar to that described by Finkel (1987). The polyols were first emulsified into the fat to be used in the chocolate formula to delay their action. It was specified that the fat used should have a solids content of 50-75% at 10°C. About 2-10% polyol along with about 1% of an emulsifier such as mono- and diglycerides were added to the fat and this mix was homogenized. It was noted that the polyols were difficult to incorporate into the fat. This emulsion was then used as the fat phase in a conventional chocolate formula. About two thirds of the fat phase was added to the other ingredients during refining; the remaining emulsion was added during conching. The final chocolate contained around 2% polyol. The chocolate could then be used for enrobing or moulding. A period of storage was required for heat resistance to develop. The product could be held at 21, 27, or 32°C for two weeks, one week, or two days respectively to achieve a chocolate which does not deform at 38°C. A very similar procedure was described by Beckett (1995).

The difficulties and extra equipment required for formation of the polyol-in-fat emulsion would be unappealing to manufacturers. Furthermore, the storage time and conditions required for heat resistance to develop represents added manufacturing costs. The chocolate formulas described in this patent’s examples contain milk solids suggesting again as described by Kempf (1958) that perhaps hydration of proteins plays a role in the development of heat resistance in chocolate. It is also clear that a sugar network could be formed in this chocolate via the hygroscopic action of polyols during the heat resistance development period.

Mandralis and Weitzeneker (2001) proposed a method which produced HRC by dispersing 0.2-60% polyol throughout the chocolate via diffusion out of gel particles. Gel particles were formed using common gelling agents (gelatin, agarose, pectin, kappa-carrageenan etc.) individually or in combination, in amounts between 0.5-15.0% using conventional gelation methods. The polyol
gel was frozen at -170 to -200°C and ground in a cold grinder to a size 1-1000 μm in diameter. An anti-caking agent such as cocoa powder was added to prevent agglomeration of the particles.

Another method of producing gel particulates was also employed. This method involved dispersing gelling agent in cold polyol and adding this to molten cocoa butter and heating to above the dissolution temperature of the gelling agent (90-170°C) with stirring. This formed an emulsion. The droplet size could be reduced by increased agitation or by homogenization. Agitation was continued while cooling and produced a mass with polyol gel droplets dispersed evenly in a solid cocoa butter matrix. The polyol gel could be added before, during or after tempering of the chocolate so long as the gel remained solid during subsequent processing. This process created HRC that was hard at 40°C however heat resistance required 3-29 days to develop depending on the formula. This “hardening time” increased with increasing concentration of gelling agent, decreased with increasing concentration of polyol gel, and decreased with decreasing gel particulate size. Furthermore, gels which contained water and polyol (water at levels of 0.66-4 times the amount of polyol) hardened in 3-5 days compared to gels which had no water (12-29 days). A very similar process was described by Best et al. (2005).

Perhaps in this instance, as with Finkel’s (1987) work, water was absorbed by the hygroscopic polyols and the water aided the development of a sugar network which resulted in the heat resistant phenomena. It should be noted that the hardening time of HRC containing no water in the gel as described by Mandralis and Weitzenockey (2001) was 12-29 days. This nearly matches the 14-28 days it takes for both O’Rourke (1959) and Kempf’s (1958) HRC to absorb enough water to develop heat resistance. As with the other systems which included water, sugar bloom may present a quality problem with this HRC. Storing these chocolates for the required hardening time would also be an expensive practice.

In a method similar to that developed by Shubiger and Rostagno (1965), Fuisz, Batist, Appl, and Richards (1994) suggested that a HRC could be formed by mixing a substantially amorphous shearform material with a hydrophobic material. For HRC specifically, a “substantially amorphous shearform material” could be sucrose formed into cotton candy via a flash heat process such as that produced by a cotton candy machine. The hydrophobic material would be
cocoa butter and/or a cocoa butter substitute. To produce HRC, amorphous shearform sugar was added to chocolate at 46.1°C and mixed for one hour. The ratio of amorphous shearform sugar to cocoa butter was in the range of 1:3 to 3:4. This ratio allowed for the HRC to be easily conched and tempered prior to moulding. The heat resistance of chocolate formulas were reported as slip points and were found to be above 79.4°C.

Some benefits achieved by this method of producing HRC included having a product that was stable at fairly high temperatures and resisted oil migration that could lead to fat bloom. In addition, the material that was added is normally found in chocolate therefore problems with food legislation may be avoided. In contrast, some detrimental aspects of this product are the need for a large amount of sugar which may adversely affect flavour and textural properties of the resulting chocolate. This article did not put forth any data on the sensory evaluation of its product. Furthermore, the sugar structure created was found to be delicate, unstable and difficult to implement in a commercial setting (Davila & Finkel, 2002). There is also an argument that water is somehow involved in this method of HRC manufacture. It has been reported that the higher the amount of amorphous sugar present in this chocolate, the higher its water content (Mathlouthi & Reiser, 1995). Amorphous sucrose was listed as a humectant which could be used in O’Rourke’s (1959) method to produce chocolate that is heat resistant after it has absorbed a certain amount of water. Therefore, perhaps a water-mediated mechanism of producing HRC is at play here.

**Incorporating encapsulated water.** Giddey and Dove (1984) prepared HRC by introducing a small amount of water to chocolate via diffusion out of a water in oil (W/O) emulsion. The emulsion was created by adding 30-80% water and an emulsifier to fluidized cocoa butter (containing at least 20% solid fat) under vigorous mixing in an emulsifying apparatus. Water droplets were reported to be 0.1-100 μm in diameter. This emulsion was either made into a thick cream or cooled so that the fat solidified. Once cooled, the emulsion could be ground into a powder, stored, and eventually supplied to users. The emulsion was added to conched chocolate, tempered or not, at levels of 2-10%. This amount of emulsion ensured the incorporation of 1-4% water into the chocolate. It was found that the emulsion particles acted as crystallization seeds eliminating the need for a separate tempering process. One important aspect of this method was to ensure that the chocolate mass, to which the emulsion was added, was at 29-31°C to prevent
early release of the water due to melting of the fat phase. Similarly, prolonged mixing of the chocolate and emulsion need be avoided to prevent breaking of the hydrophilic network. This process was similar to the method described by Rosenthal, Pikalla, Cook, and Korfhage (1966) however, these researchers did not report observations of a heat resistant property in their W/O chocolate emulsion.

Heat resistance of the aforementioned chocolate was achieved after solidification of the chocolate and was found to increase with time. Heat resistance of the chocolate was tested by incubating the samples at 40°C for 2 hr and thereafter performing a penetration test with a vertical needle. The needle applied an increasing force to the surface of the chocolate until 3 mm penetration was achieved. As shown in figure 2.2 heat resistance of chocolate manufactured via Giddey and Dove’s (1984) method was much greater than that of conventional chocolate and was dependent on the type of chocolate used. Once released, the water in this chocolate likely facilitated the formation of a sugar network. This network provided the chocolate structure and the ability to resist penetration at elevated temperatures even though the fat had melted.

This method for production of HRC did not require a waiting period for the development of heat resistance and produced chocolate which could withstand temperatures near 50°C. The method, excluding the emulsion production, was quite simple and it is plausible that chocolate of this type could be created in a home setting. The chocolate produced was also said to have a taste and feel similar to conventional chocolate however no sensory evaluation data was given. The excess fat found in this chocolate would likely alter some sensory characteristics. Furthermore, as with any system that introduces water to chocolate there is likely a risk of sugar bloom development resulting in surface and/or textural defects (Timms, 2003).
Kealey and Quan (1992), Takemori, Tsurumi, and Takagi (1992), Traitler, Windhab, and Wolf (2000), and Simbürger (2009) developed similar methods to those above. Kealey and Quan (1992) found that adding about 0.3% protein in the form of sodium caseinate or skim milk powder to the W/O emulsion led to decreased phase separation and thus a more stable emulsion. Takemori, Tsurumi, and Takagi (1992) used a solution of saccharides in water as the dispersed phase of their W/O emulsion; the emulsion containing 20-60% saccharides, 10-50% water, 30-60% fat, and 0.1-3% emulsifier. It is possible that the saccharides added as part of this emulsion would aid in the formation of the sucrose network. Simbürger (2009) added 1.8-7% water to conventional chocolate by adding the water directly, as an O/W emulsion or preferably as a W/O emulsion. Similarly to Takemori, Tsurumi, and Takagi (1992), Simbürger (2009) added 30-75% hydrophilic substance (such as saccharides) to the W/O emulsion. The chocolate mass with added water was then moulded and subsequently subjected to a microwave treatment for 5 s to 6 min to remove some of the water. The length of the treatment was found to depend on the dielectric constant, size, and shape of the chocolate. The microwave treatment eliminated lengthy storage time required for heat resistance to develop. It was found that it was necessary to cool the exterior of the chocolate during the microwave treatment to prevent loss of shape or fat bloom. The interior of the chocolate was required to reach 90-135°C for heat resistance to be achieved. Milk chocolate burns at temperatures above 85°C (Mohos 2010) therefore this microwave treatment would likely cause some burning of the chocolate. Furthermore, the equipment necessary for the microwave treatment would be costly to manufacturers (Potter & Hotchkiss 1998).
Alander, Wärnheim and Lühti (1996) modified the above methods by producing HRC via the incorporation of a W/O microemulsion. Water droplets formed by this method were claimed to be 0.001-0.1μm in diameter. The microemulsion could have various formulations but examples given were made of around 60% emulsifier, 15-20% water, and 20-25% fat. The microemulsion was added at levels of 2-17% by weight to conventional chocolate, mixed, and moulded. The final chocolate would therefore contain approximately 0.4-3.4% water. The chocolate, after solidification, was able to hold its shape at temperatures up to 40°C.

This method, although similar to those above, had some added benefits. The spontaneous formation of thermodynamically stable microemulsions eliminated the extensive mixing and homogenization processes that are necessary to form emulsions (Hubbard, 2002). Furthermore, it was proposed that the smaller water droplet size made for finer distribution of the water leading to HRC being produced with less water added. The lesser amount of emulsion added to the chocolate would mean that a lesser amount of fat was added to the system. This was advantageous because excess fat could alter sensory characteristics and could make heat resistance more difficult to achieve due to the fat being liquid at elevated temperatures. However, this microemulsion did contain much more emulsifier than those described above which could limit its addition to chocolate due to food legislation restrictions (Canada, 2009). Furthermore, the only reported measure of heat resistance given was that the chocolate was able to hold its shape up to 40°C which is a temperature much lower than what was achieved by other methods including that by Giddey and Dove (1984). The incorporation of water could also pose a problem with sugar bloom (Timms, 2003). Lastly, sensory evaluation of the product would be necessary to ensure the chocolate was acceptable to consumers.

In another attempt to modify the method developed by Giddey and Dove (1984), a method was developed by Frippiat and Smits (1996) that used inulin gel to introduce water into chocolate. The inulin gel was prepared by mixing 50% inulin with water under high shear such as that provided by a homogenizer. About 5% of this gel was added to conventional tempered milk chocolate and mixed. There was no indication as to the degree of heat resistance achieved via this method. The addition of inulin to chocolate will likely have restrictions put in place by food regulations in some countries (Beckett 2000).
Frippiat, De Soete, Smits and Keme (2000, 2005), in a process similar to that proposed by Mandralis and Weitzenecker (2001), encapsulated water in a gel and added the gel to chocolate. The gels used were prepared by conventional procedures and could be made from carbohydrates or pectins among other materials. The gels were then simply mixed with chocolate prior to or after tempering and the chocolate was left to set. The amounts of ingredients used varied depending on the gelling agent and type of chocolate. Final chocolate formulations contained 4-18% gel giving a moisture content of 2.5-8% water. It was found that the resulting chocolates were able to hold their shape even after six hours at 40°C and did not melt or feel sticky when touched. Some of the chocolate tested was able to hold its shape up to 75°C. Although no sensory evaluation data was given, it was reported that at least some of the HRC made was acceptable or retained organoleptic characteristics similar to conventional chocolate. In addition, to observe the formation of surface defects the chocolates were left in a chamber at 20°C and 65% relative humidity for 15 days. The surface of the chocolates remained unchanged during that time.

This chocolate displayed some heat resistance abilities. However, food legislation in some countries may limit or prohibit the addition of the gelling agents to chocolate (Canada, 2009). Many of the gels used in the examples given had a cream-like consistency and were easy to incorporate into chocolate. However, more solid gels may require a further processing step to break the gel into tiny pieces prior to their addition to chocolate. Supplementary equipment and processing would add to the cost of manufacture. The initial test to monitor the development of surface defects showed promising results. Further testing should be completed at higher temperatures to mimic the environments the HRC may be subjected to in summer or tropical climates. This would indicate if the water in the chocolate would cause sugar bloom under these conditions.

2.2.1.2 Development of a stable, jammed particle network
In a method described by Crosley and Conner (1945), HRC was developed by a method of “cold-working” a conventional chocolate formula. The ingredients including 26-29% cocoa butter, 12-24% cocoa solids, 30-50% sucrose, and 0-20% skim milk powder were either premixed and allowed to solidify prior to cold-working or simply combined during cold-working. The mix was passed through opposing rollers or similar equipment at a temperature not
exceeding 28.3°C or the temperature at which the fat phase melts. It was suggested that the
temperature could be maintained by passing refrigerant through the equipment during
processing. The mass may need to pass through the rollers several times. The finished chocolate
composition was then scraped from the rolls as broken fragments of a thin sheet which tended to
be curled or rolled up. Initially the chocolate was waxy and flexible but within about a minute it
became dull and brittle. The chocolate could then be shaped by extrusion or tableting. These
processes occurred without delay after cold-working to ensure the chocolate was cohesive and
retained its shape. The chocolate resisted softening after 1 hr at 48.8°C. Researchers believe that
heat resistance in this chocolate was achieved by the redistribution of the continuous fat phase
into dispersed globules finely divided by the remaining ingredients. This is similar to what was
observed by Zizinia and McKenna (1940) and their powdered chocolate liquor. This method of
HRC manufacture would require specialized equipment. Furthermore, the product was limited to
extrusion or tableting for shaping and it could not be used for enrobing. It was also noted that the
chocolate composition tended to adhere to the rollers during cold-working which could be
problematic.

Similar technology was described in a Swiss patent by Despland and Guinard (1972). Here it was
mentioned that gelled starch or gelatin could be added to the chocolate to aid in the encapsulation
of the fat phase.

2.2.1.3 Development of a secondary network of high melting point emulsifier
A HRC product was developed by Nalur and Napolitano (2002) by addition of a high melting
point emulsifier to conventional chocolate. The emulsifier used had a melting point from 50-
90°C, a hydrophilic lipophilic balance (HLB) of 2-10 and was added at levels of 1-6% of the
chocolate (or 3-15% of the fat phase). Examples of emulsifiers which fit these properties include
diacetyltartaric acid ester of monoglycerides (DATEM), sorbitan esters, and mono- and
diglycerides. The emulsifier could be added to the other ingredients and mixed thereafter at
anytime during the conventional chocolate manufacturing process. However, it was required that
the chocolate be at a temperature near or above the melting point of the emulsifier being added.
The molten chocolate with emulsifier was then be deposited into moulds and cooled. The
resulting chocolate could maintain its structure up to 45°C. It was found that the heat resistance
achieved was affected by the type of emulsifier used, especially the chain length of
monoglyceride used. Generally, emulsifiers with longer chain length (and therefore higher melting temperature) showed stronger structure within chocolate at elevated temperatures. Furthermore, the heat resistance was also dependent on the fat phase used. An informal sensory panel found that chocolate made with 3% monoglycerides (MP 67°C) was not perceived as waxy but was perceived as more fatty than chocolate without the emulsifier. The sample chocolate was also perceived as creamy with an acceptable texture. The mechanism of heat resistance was believed to be the result of the formation of a rigid emulsifier structure in the chocolate that physically interacts with the liquid oil molecules. The emulsifier thus traps the fat within the chocolate at elevated temperatures. This is similar to the aforementioned sugar networks trapping the molten fat in the chocolate when the temperature increases above the melting point of the fat.

This method proved somewhat successful at producing HRC however much of the empirical evidence of heat resistance was ambiguous. One key concern with this method was the inability to temper the chocolate due to the high temperature required prior to moulding to keep the emulsifier from crystallizing. This would limit the ability of manufacturers to use cocoa butter as the fat phase for this chocolate. Furthermore, food regulations could limit the amount and type of emulsifiers used in this chocolate (Canada, 2009). This method was advantageous in that no special equipment was required, there was no development time necessary for heat resistance to be obtained, and initial sensory tests found the chocolate to have acceptable texture. It is a concern that the high melting emulsifiers used in this method may give a waxy feeling to the chocolate. A formal sensory panel would be necessary to eliminate this concern.

2.2 Addition of an oil/fat binding polymer

In an early patent by Logan (1939), a chocolate product was made by mixing together 25.8% fat, 16.3% cocoa powder, 35.5% sugar, 15.5% skim milk powder, and 6.6% raw oat flour. Oat flour contains approximately 80% starch, 6.8% protein, and 0.5% β-glucan as well as some moisture, lipid, and ash (Lim, Liang, Seib, Rao, 1992). The ingredients were thoroughly mixed using finishing rollers. The resulting putty-like substance was forced into moulds to obtain the desired shape. It was found that at elevated temperatures the combination of skim milk powder and oat flour absorbed the liquid fat allowing the chocolate to maintain its shape up to 54.4°C. It was found that chocolate made in this manner had poor palatability, had a porous structure, and the viscosity was very high such that the chocolate was difficult to mould.
A similar method of producing HRC was recently developed by Ogunwolu and Jayeola (2006). Cornstarch or gelatin was added at levels of 2.5, 5.0, 7.5, or 10.0% to other chocolate ingredients at the grinding and mixing stage of conventional manufacture. If used, gelatin was added during the latter part of mixing. A Gallenkamp melting point apparatus was used for measurements and determined that an increase in the amount of starch or gelatin resulted in an increase in melting point as shown in figure 2.3. It was found that the moisture content of chocolate made with cornstarch increased with increasing amounts of cornstarch. This extra moisture is a possible explanation as to why cornstarch produced chocolate with higher melting points than gelatin as previous examples have shown that water has the ability to change the heat resistance of chocolate (Russell & Zenlea, 1948; Kempf, 1958; O’Rourke, 1959). Sensory evaluation showed that chocolate made with 10% cornstarch was not significantly different (p < 0.05) than conventional milk chocolate in colour, taste, smoothness, and overall acceptability. However, there was a significant decrease in sweetness in the chocolate made with cornstarch. Chocolate made with 10% gelatin was not significantly different than conventional milk chocolate in colour, sweetness, and overall acceptability. However, the chocolate made with gelatin was found to have significantly poorer taste and decreased smoothness when compared to conventional milk chocolate.

![Figure 2.3 Melting points of chocolate with addition of cornstarch or gelatin. Adapted from Ogunwolu and Jayeola, 2006](image)

The addition of oat flour, gelatin or cornstarch to chocolate was successful in increasing its heat resistance. The mechanism that produced heat resistance in these chocolates was not explained by the researchers. The polymers added (starch, milk proteins, β-glucan, and gelatin) caused an
increase in the viscosity of the chocolate. Perhaps this increase in viscosity was responsible for chocolate’s ability to maintain its shape at elevated temperatures. It is likely that the polymers formed a network which physically trapped the fat from leaking out of the chocolate at elevated temperatures. These polymers are hydrophilic and do not bind much oil therefore it is unlikely that chemical bonding of the oil to the polymer is occurring in these samples.

Relatively large amounts of these ingredients were needed to achieve heat resistance. Food legislation in some countries in the world does not permit any amount of oat flour, cornstarch or gelatin to be present in chocolate (Canada, 2009). This will limit the applications of these methods of producing HRC. The chocolate produced was found to be acceptable by sensory evaluation; however, some characteristics of the thickened chocolate were found to be less desirable than those of conventional chocolate. One particular disadvantage to these methods was that the increased viscosity of the resulting chocolate paste made it sometimes difficult to work with and mould. This could cause problems with manufacturing equipment, necessitating more powerful pumps and may decrease the quality of the chocolate produced.

Recent work by the authors has revealed a new and novel method of preparing HRC. The chocolate was produced by mixing a solution of the polymer ethylcellulose (EC) in ethanol with conventional chocolate (milk, dark, or white) after tempering. The chocolate was mixed until homogeneous, moulded, cooled and then subjected to a drying treatment to evaporate the ethanol. The most advantageous EC in ethanol mix was a 20% solution of EC cP 10. This solution was added at levels around 10% to the chocolate such that when dry, the chocolate contained around 2% EC. The chocolate was stored at 30°C for 9 days to evaporate the ethanol. The resulting chocolate resisted deformation at temperatures >86°C (see figure 2.4). Multiple variables including the molecular weight of the EC used, amount of EC added and ratio of EC to ethanol in the mix could be altered to achieve various degrees of heat resistance. The resulting HRC made by this method is a chocolate that maintains structure at elevated temperatures and does not oil off.

Preliminary experiments into the mechanism of heat resistance in this chocolate have revealed an important interaction between the EC and sugar in the chocolate. The authors speculate that perhaps the EC assists the sugar in forming a network that traps the fat at elevated temperatures.
Furthermore, the ethanol also likely plays a key role in building this sugar network. Phosphatidylcholine (PC), one of the major phospholipids responsible for the surfactant properties of lecithin, is soluble in ethanol (Teberikler, Koseoglu, and Akgerman, 2001). In chocolate, lecithin is added to reduce the viscosity by coating the sugar molecules in such a way that the continuous fat phase easily flows over their surfaces. The ethanol from the EC solution likely removes some of the PC from the surface of the sugar molecules thereby exposing this surface for network formation. The EC in this chocolate is likely also responsible for some oil binding. It has been shown that EC has the ability to gel oil when added at levels of >2% to oil, heated to above the glass transition temperature of the EC (~145°C) and subsequently cooled (Aiache, Gauthier, and Aiache 1992; Gauthier, Aiache, and Aiache 1994; Howard 1976). Perhaps the dissolution of EC in ethanol relaxes the polymer in much the same way that is observed when the polymer is heated above its glass transition temperature. After addition to the chocolate the relaxed polymer undergoes a solvent substitution where the ethanol that is evaporated off and is replaced by the fat in the chocolate. Further research is required to confirm these hypotheses.

![Figure 2.4](image)

Figure 2.4 Heat resistance of chocolate measured after 2 h at specified temperature with large deformation machine and cylindrical probe. Compound milk chocolate was used as a control; HRC contained compound milk chocolate with 2.22% EC cP 45 incorporated from a mix of 20% EC in anhydrous ethanol

This method of producing HRC is quite simple and effective. However, food legislation in some countries may limit or disallow the addition of EC to chocolate (Canada 2009). Furthermore, the
use of ethanol and the time required to remove it from the chocolate is not ideal and could pose a danger in the production facility.

2.2.3 Increasing the melting point of the fat phase

Increasing the melting point of the fat phase is a well known method of producing HRC. The use of interesterification or addition of a fat with a higher melting point are two ways in which this can be achieved. Specific examples of these methods are outlined below.

2.2.3.1 Interestenification of the fat phase

The well known practices of chemical and enzymatic interesterification of fats were used by Bruse, Wallecan and Arruda (2008) to provide heat and fat bloom resistance to chocolate. Interestenified (IE) cocoa butter (CB) with a melting point above conventional CB was produced by adding lipase or any suitable enzyme in immobilized or free form in an amount of 0.01-10% of enzyme to CB. The increase in CB melting point was further enhanced by using directed interesterification. When interesterification is carried out below the melting point of higher melting (saturated) triacylglycerol species, as occurs during directed interesterification, there is an enrichment of such high melting species in a mixture of triacylglycerols (Fennema, Damodaran, & Parkin, 2008). Fractionation, dry or solvent, was optionally used to further increase the melting point of the CB produced. This fully or partially IE CB was then added as a portion of the fat phase to the other chocolate ingredients prior to conching and production was continued in the conventional manner. The final fat phase of the chocolate produced included 1-30% IE CB, 45-99% unmodified CB, and 0-20% milk fat by weight. It was found that the slip melting point of CB that was interesterified using 2% lipase at 70°C for 6 h increased from 26°C to 42.5°C with an increase in free fatty acid content from 2.1-2.7%.

Figure 2.5 (A) shows that slip melting points of the chocolate were dependent on the amount of IE CB used to replace CB. Specifically a chocolate containing 30% IE CB and 70% unmodified CB was around 37°C depending on the type of CB used. Sensory evaluation, using a triangle test, showed that 13 out of 14 panelists were able to identify a chocolate sample containing IE CB as different from an unmodified sample. The IE sample was preferred for its smoothness, creaminess, and softness when compared to conventional chocolate. Another sensory test with 45
panelists showed that the appearance and mouth-feel of a sample containing 6.6% IE CB was equal to a reference with no IE CB.

Figure 2.5 Addition of higher melting fats to improve the heat resistance of chocolate. (A) Slip melting points of chocolate with cocoa butter (CB) replaced with interesterified CB (adapted from Bruse, Wallecan and Arruda, 2008). (B) Melting profiles of CB, mahua fat, and kokum fat and various combinations thereof (adapted from Jeyarani and Reddy, 1999). (C) Hardness of chocolate with CB replaced with kokum fat (tested at 30°C) (adapted from Maheshwari and Reddy, 2005)
Overall this method of increasing the heat resistance of chocolate is quite advantageous. The product created had an immediately increased melting point. Furthermore, sensory evaluation of the IE product showed some positive results. However, overall preference or liking of the IE product was not tested. The increase in free fatty acid content following IE was small but there could be a risk of an increase in oxidation products leading to rancid or off-flavours in the chocolate (Fennema, Damodaran, & Parkin, 2008). Finally, as with previously mentioned methods, the use of enzymes is quite expensive however, the ability for this method to use immobilized enzymes offered a way to decrease this cost (Fennema et al.).

2.2.3.2 Addition of a high melting point fat

Another approach to increase the melting point of the fat phase of chocolate developed was to incorporate fats with higher melting points. One specific example of this was studied by Jeyarani and Reddy (1999) and focused on using mahua (Madhuca latifolia) and kokum (Garcinia indica) fats to increase the melting point of a cocoa butter blend. Mahua and kokum are both trees found in several parts of India. The kernels found in the fruits of mahua trees contain semi-solid fat. Conversely, kokum kernels contain a hard, brittle fat with a melting point 39-43°C. The products of fractionation and blending these fats were evaluated for their ability to increase the melting temperature of and replace the fat phase in chocolate products. Fractionation was used to separate the stearin fraction from kokum and mahua fats because it was reported that addition of fats rich in 2-oleodistearins to cocoa butter can increase the solid fat content (SFC), increase the melting point and decrease the tempering time of chocolate (Jeyarani & Reddy 1999). Both solvent and dry fractionation methods were used. Using the solvent fractionation method, the stearin fraction of mahua fat was obtained (35% yield) and then mixed with kokum fat in a 1:1 ratio. This blend was solvent fractionated again leaving the stearin fraction (F1) of the mahua and kokum blend (77-80% yield). For dry fractionation mahua and kokum fats were mixed in equal proportions, heated to 55°C, gradually cooled to 27°C, and held at this temperature for 2 h with occasional stirring. The stearin fraction (F2) was separated by filtration (yield 77%).

Mahua fat alone, once fractionated, did not show physical or chemical characteristics that were acceptable to improve the heat resistance of CB. However, by blending mahua and kokum fats, improved solidification and melting characteristics were achieved. As seen in figure 2.5 (B) it was found that at 32.5°C F1 and a blend of F1 with CB had higher SFC than CB. A decrease in
the amount of F1 in the CB blend led to a decrease in the SFC. Fraction 2 which was obtained by dry fractionation showed characteristics similar to F1. However, F2 was softer than F1.

Additional studies were carried out to analyze the characteristics of blends of mahua fat or mahua stearin and kokum fat without further fractionation. Blends of 40:60 mahua fat or mahua stearin with kokum fat showed SFC similar to F1 although were slightly softer. However, non-fractionated fat blended with CB showed lower SFC compared to blends of F1 and CB. Results showed that blends containing 50:50 mahua fat or mahua stearin and kokum fat also exhibited the abilities to increase the SFC of cocoa butter although these blends were somewhat softer than F1 and the 40:60 blends. It was also noted that mahua and kokum fats are miscible with each other and with cocoa butter, eliminating any unwanted eutectic behaviour.

This method successfully produced a fat phase that achieved higher SFCs at elevated temperatures than conventional chocolate. However, once the temperature reached 37.5°C the SFC of most of the blends was less than 20% indicating that the heat resistance of the chocolate would be lost at temperatures higher than this. The replacement of cocoa butter with fats from less expensive plants in chocolate represents a potential cost reducing practice (Pease, 1985). This study lacks any sensory evaluation of the resulting fat blends. Consumer acceptability of the product must determine if manufacture of a chocolate product with these fats is worth pursuing. Finally, many countries have limits as to how much non-cocoa vegetable fat (NCVF) can be added to chocolate. For instance, the US, Canada, and India allow no NCVF to be added to chocolate (Timms, 2003). However, most of the EU and Japan will allow up to 5 and 12% NCVF respectively to be added to chocolate. Therefore, the addition of NCVF such as mahua and kokum fats to chocolate as proposed by Jeyarani and Reddy (1999) has limited legal allowance.

In a study similar to the one mentioned above, Maheshwari and Reddy (2005) attempted to improve the heat resistance of chocolate via replacement of some cocoa butter with kokum fat. Kokum fat was refined but not fractionated and then blended with cocoa butter at various levels. Results shown in figure 2.5 (C) show that at 30°C the hardness of dark and milk chocolates was found to increase gradually with increased levels of kokum fat. In agreement with this finding, SFC was also found to increase with increased levels of kokum fat. Furthermore, the melting
peak temperatures also increased with an increase in levels of kokum fat with the blend containing 5% kokum fat having a melting temperature of 34.8°C. Sensory evaluation was carried out with 15 trained judges on samples of milk and dark chocolate containing up to 5% kokum fat. Results showed no significant differences in gloss, snap, finger print resistance, melt-in-the-mouth properties, and overall quality compared to a control chocolate with no kokum fat.

This method to produce a more heat resistant chocolate has similar advantages and disadvantages to the aforementioned example. The chocolate showed good sensory properties. However, the heat resistance of this chocolate is not excellent considering the melting temperature is only 34.8°C with 5% inclusion of kokum fat. Finally, the chocolate formulas developed exceed some countries’ legal limits for inclusion of NCVF (Timms, 2003).

2.3 Conclusions

Through this literature review it is evident that a heat resistant chocolate can be made. However, none of the examples illustrate a simple, inexpensive, and successful HRC. It is obvious that the quality of HRC is lower than that of traditional chocolate. It is surprising that so many attempts have been made to incorporate water into chocolate while during conventional chocolate processing water is purposefully avoided. For future studies on this subject, it is recommended that water be eliminated from any formula or method developed. Furthermore, heat resistance was poorly defined in most of the articles reviewed. Better comparisons could be made amongst articles reviewed if there was a standard measure of chocolate heat resistance. More research needs to be carried out and reported in peer reviewed journals so that a better understanding of previous attempts to produce HRC can be reached. Specifically, it would be helpful to understand how polyols contribute to heat resistance.

As mentioned previously, chocolate standards of identity are very strict (Timms, 2003). Therefore, it would be advantageous to produce HRC with a formulation that is least altered from conventional chocolate. Many of the methods described above utilize only ordinary chocolate ingredients plus water in their formulas including the incorporation of a W/O emulsion into chocolate. This method was first described by Giddey and Dove (1984) and subsequently refined by Kealey and Quan (1992), Takemori, Tsurumi, and Takagi (1992), Alander, Wärnheim, and Lühti (1996), Traitler, Windhab, and Wolf (2000), and Simbürger (2009). The
main benefit of producing HRC in this manner was that by encapsulating the water it was physically separated from the sugar in the chocolate and therefore could not dissolve the sugar. This chocolate was therefore not prone to increases in viscosity and the resulting difficulty in manufacturing as was observed with many of the other methods discussed. Furthermore, if the W/O emulsion was made with cocoa butter as the oil phase and a traditional chocolate emulsifier then the resulting chocolate would contain only conventional chocolate ingredients plus 1-4% water. Giddey and Dove (1984) were able to achieve immediate and good heat resistance with just a small amount of water. Homogenization of the water-in-oil emulsion described in this method would add to the cost of manufacture. However, after the emulsification process the HRC was quite simple to produce. In our view, Giddey and Dove (1984) have developed the most promising method of producing HRC thus far.

References


2.4 Additional Literature

A summary of additional literature on heat resistant chocolate not included in the original published version of *Heat resistant chocolate* is presented here.

Thiele, Paggios, and Balzer\(^1\) (2012) developed a method similar to that developed by Lataner\(^2\) where water was directly incorporated into chocolate to provide heat resistance. The water was sprayed at levels of 5-35% by weight onto conventional chocolate typically in a multi-layered fashion. Optionally, the chocolate could also be sprayed either in alternating layers with the water or simultaneously with the water. This would be useful for enrobing products in chocolate. Unfortunately, chocolate to be sprayed was required to have a very low viscosity preferably from 0.5-3.0 Pa·s. This could be achieved by addition of large amounts of emulsifier; typically addition of emulsifiers has a negative impact on heat resistance of chocolate. This process could also be modified by using a mixture of water and polyol or polyol alone all of which have the ability to dissolve some of the sugar present in the chocolate to create a sugar network capable of providing heat resistance. The chocolate produced in this manner was reported as being not sticky at temperatures up to 50°C.

Galdón Miquel et al.\(^3\) developed heat resistant chocolate using methods very similar to Zizinia and McKenna\(^4\) where a typical chocolate formula was homogenized with 30% water and then dried to <5% moisture. Though the chocolate produced did not melt at temperatures above 40°C it was in the form of powder, scales, or flakes and there was no mention of how to use this chocolate for enrobing or forming into bars or shapes.

Beckett et al.\(^5\) developed a milk chocolate that incorporated encapsulated water as a water-in-oil emulsion similar to the method by Giddey and Dove\(^6\). Dark chocolate was added to the emulsion with light mixing for 1-10 min at 32-50°C. Thereafter, the milk powder was added as a suspension in liquid fat (or oil) and again mixed gently with the other ingredients. It was very important for the chocolate to be subjected to minimal shear such that the emulsion didn’t break. For this reason the seed method of tempering was required. The chocolate produced contained 1.5-25% water. This chocolate was not described as heat resistant though it likely has some heat resistant properties.
Recently, Killian and Coupland\(^7\) studied model chocolate systems to try to understand how addition of water, particularly in the form of water-in-oil emulsions can induce heat resistance in chocolate. They found that when water was added to a system of sucrose crystals in oil the water was found at the surface and between adjacent sucrose crystals. The water accumulated in these capillaries between crystals to reduce its surface curvature thereby creating an attractive force between the sucrose particles. Furthermore, the water would dissolve some of the sucrose increasing the viscosity of the liquid in the capillaries and leading to even better cohesion. Finally, if the water evaporates it would leave behind recrystallized sucrose bridges which would be three times stronger than the liquid capillaries. Liquid polyols which can dissolve some of the sucrose would probably also form capillaries and sucrose bridges via the same mechanism as water. It is likely that these capillaries and recrystallized sucrose bridges are responsible for the heat resistance achieved in many of the chocolate formulas developed which seek to incorporate water or polyols into chocolate.

Schlup and Lioutas\(^8\), Wang and Hickey\(^9\), de la Harpe and Dickerson\(^10\), and Silvano and Dhami\(^11\) have all produced heat resistant chocolate by methods which ensure the sucrose particles present do not become fully coated in fat. This method was first introduced by Friedman.\(^12\) Schlup and Lioutas\(^8\) combined the use of unconched chocolate with the addition of 1-16% water to produce heat resistant chocolate. Wang and Hickey\(^9\) produced heat resistant chocolate by simply not conching a conventional chocolate formula. Similarly, Silvano and Dhami’s\(^11\) method used >50% unconched chocolate mixed with conched chocolate to achieve heat resistance. Advantageously, by combining conched and unconched chocolate this method was also able to achieve the desirable flavours of typical chocolate which are developed during the conching process.\(^13\) These methods were slightly modified by de la Harpe and Dickerson\(^10\) who followed the normal chocolate manufacturing scheme of refining and then conching the chocolate but then the conched chocolate was “re-refined” to break up the sucrose particles again and create non-fat coated sucrose surfaces. Again, this method was advantageous because including a conching process allowed for the proper flavour development to take place. It is believed that heat resistance in all of these chocolates was achieved by the uncoated sucrose crystals interacting with one another to form a network throughout the chocolate that provided structure when the fat melted.
Many of the newer methods were clearly very similar to the heat resistant chocolate methods developed decades ago. Some of the methods provided new advantages such as the ability to have a finished chocolate flavour in a non-conched chocolate. However, others have introduced multiple new processing steps and the use of unconventional equipment which tend to complicate and add cost to the chocolate making process. Nonetheless, the new patents reiterate that many international chocolate companies are still trying to perfect, and bring to market, heat resistant chocolate.

References

4 *US Pat.*, 2 201 820, 1940.
12 *US Pat.*, 1 364 192, 1921.
CHAPTER 3
Ethylcellulose Solvent Substitution Method of Preparing Heat Resistant Chocolate

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Abstract
In the present study a structuring technique was developed to produce chocolate which resists deformation at temperatures above 40°C. It was hypothesized that by adding ethylcellulose (EC) solubilised in ethanol (EtOH) to chocolate and evaporating the EtOH an organogel could be formed in situ with the fat phase of the chocolate. Heat resistant chocolate (HRC) was produced by mixing a 20% EC in EtOH solution with molten chocolate. The EtOH was evaporated and the resulting chocolate was incubated at 40°C for 2 h and tested for hardness. The effect of various EC viscosities (4, 10, 20, 22, and 45 cP) and concentrations ranging from 1.0 - 2.2% on different types of chocolates were studied. Milk chocolate containing 1.9% EC had a hardness of 26.0 N whereas the control chocolate was too soft to be tested. Further experiments revealed that white and dark chocolates had hardnesses of 29.5 and 10.5 N, respectively. The hardness of the chocolate was dependent on the chocolate formulation and concentration of EC, and independent of EC viscosity. It was observed that addition and evaporation of EtOH from the compound milk chocolate samples led to an increase in the lightness of the chocolate surface if the EtOH was evaporated at temperatures of 40°C or higher. Addition of EC to chocolate represents a new strategy for the manufacture of HRC. Future work should focus on determining the mechanism by which heat resistance is achieved in these chocolates.

3.1 Introduction
Chocolate is a widely loved treat that has been enjoyed for centuries. Worldwide chocolate sales have surpassed US$90 billion (Schmitz & Shapiro, 2012). This confection is known for its desirable melt-in-your-mouth characteristic, a phenomenon attributed to the melting point of cocoa butter (34°C) (DeMan, 1999), the main fat constituent in chocolate, being just slightly below body temperature (37°C). However, this melting point is problematic when manufacturing
or selling chocolate at ambient temperatures close to or above this temperature such as occurs in summertime or tropical climates. A chocolate that resists melting and deformation at temperatures above 34°C is therefore advantageous in these situations.

Various efforts have been made in the past to develop heat resistant chocolate (HRC), details of which can be found elsewhere (Stortz & Marangoni, 2011). Three main strategies to produce HRC have been identified: enhancing network microstructure, addition of an oil binding polymer, and increasing the melting point of the fat phase. Currently, none of these strategies has been successfully commercialized, thus further research is necessary. Of particular interest is advancement in the development of HRC utilizing polymer organogelation.

Gels in which the liquid phase is oil are classified as organogels (Marangoni & Garti, 2011). Ethylcellulose (EC) has been shown to have oil gelling abilities when added at levels of ≥ 2% by weight to oil (Aiache, Gauthier, & Aiache, 1992; Gauthier, Aiache, & Aiache, 1996; Howard, 1976) and heating above the glass transition temperature of the EC which is around 145°C (Dey, Kim, & Marangoni, 2011). This polymer is a derivative of cellulose in which some hydroxyl groups on the glucose monomers are substituted with ethoxyl groups.

The use of EC to gel the fat phase of chocolate may represent a novel strategy to produce HRC. At elevated temperatures the EC gel network could trap liquid cocoa butter and prevent collapse of the chocolate structure. However, using EC to gel the fat phase of chocolate is problematic since the typical method to create an EC gel requires the ingredients to be heated to around 145°C for a gel to form and chocolate should never be heated this high. The purpose of this study was to develop a low temperature method to introduce EC into chocolate such that heat resistance was incurred, and to characterize and optimize this HRC. It was hypothesized that a good solvent for EC such as ethanol (EtOH) could be used to solubilize the EC at room temperature. This mix could then be added to chocolate and the solvent could be evaporated leaving behind the EC where it could form a gel with the fat phase of the chocolate in situ. This method would thereby avoid the high heat normally associated with the formation of EC oleogels by employing the use of a volatile solvent.
3.2 Materials and Methods

3.2.1 Materials

Samples were prepared using commercially available chocolates. Milk, dark, and white chocolates were attained from Barry Callebaut (Chicago, IL). Compound chocolate refers to chocolate made with hydrogenated palm kernel oil (PKO) instead of cocoa butter. Compound chocolate was acquired from Bulk Barn (Richmond Hill, ON). A list of the types of chocolate used in this study can be found in table 3.1.

<table>
<thead>
<tr>
<th>Chocolate type</th>
<th>Product name</th>
<th>Ingredients</th>
<th>% fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound milk</td>
<td>Compound milk chocolate wafers</td>
<td>Sugar, hydrogenated palm kernel oil, cocoa powder, powdered whey protein concentrate, whole milk powder, soya lecithin, vanilla</td>
<td>31</td>
</tr>
<tr>
<td>Compound dark</td>
<td>Compound dark chocolate wafers</td>
<td>Sugar, hydrogenated palm kernel oil, powdered whey protein concentrate, cocoa powder, chocolate liquor, black cocoa powder, soya lecithin, vanilla</td>
<td>31</td>
</tr>
<tr>
<td>Milk</td>
<td>Kenosha milk chocolate</td>
<td>Sugar, cocoa butter, whole milk powder, chocolate liquor, nonfat dry milk, butteroil, soya lecithin, vanilla extract</td>
<td>34.5</td>
</tr>
<tr>
<td>Dark</td>
<td>Tulsa dark chocolate</td>
<td>Chocolate liquor, sugar, cocoa butter, soya lecithin, vanilla extract</td>
<td>34.1</td>
</tr>
<tr>
<td>White</td>
<td>Ultimate white chocolate chips</td>
<td>Sugar, cocoa butter, whole milk powder, nonfat dry milk, milkfat, soya lecithin, vanilla extract</td>
<td>27.9</td>
</tr>
</tbody>
</table>

EC Standard Premium grade polymers of various viscosities were attained from Dow Wolff Cellulosics (Midland, MI). The viscosity of the EC was determined by the manufacturer by dissolving 5% EC in a mix of 80% toluene and 20% ethanol (EtOH) and testing using an Ubbelohde viscometer at 25°C. EC with viscosities of 4, 10, 20, or 45 cP were used. EC 22 cP was obtained from Sigma-Aldrich (Oakville, ON); the viscosity of EC 22 cP was measured in the same manner as above. Absolute EtOH was obtained from Commercial Alcohols (Brampton, ON) and was stored in a refrigerator (5°C) with a desiccant in the bottle to minimize absorption of water. The EC in EtOH mix was prepared by slowly adding 20 or 25% EC to EtOH with
stirring in a sealable container. This mix was left overnight and stirred again to ensure full
dissolution and homogeneity.

### 3.2.2 HRC Preparation

Compound chocolate was melted using a microwave in short time increments with stirring
between heating until the mass was 40-50°C. When the mass reached 40°C the EC mix was
added quickly and the mass was stirred until homogenous or for 1 min. The sample was then
poured into room temperature moulds. Once filled, the mould was tapped for 15 s on the counter
to remove air bubbles from the chocolate. The mould was then placed in a refrigerator (5°C) for
20 min. Once hardened, the sample was removed from the mould by tapping the mould on the
counter.

To prepare HRC made with chocolate containing cocoa butter, the chocolate was first tempered
using a seed tempering method and a Revolation 2 Chocolate Tempering Machine
(ChocoVision, Poughkeepsie, NY). The seed chocolate was produced using the table top
tempering method. Chocolate was melted slowly in a microwave until a temperature around
40°C was attained. Approximately one third of the melted chocolate was poured onto a cool,
thick, metal table. The chocolate was spread out then folded back into a mound using a flat,
metal spatula. This was repeated until some of the chocolate showed signs of crystallization
which was visible as patches of lighter, thicker, and less shiny chocolate. The chocolate was
added back to the rest of the warm chocolate and stirred. The spreading, folding and
reincorporation steps were repeated until the chocolate reached its working temperature (31°C,
30°C, and 28°C for dark, milk and white chocolate respectively). If the chocolate temperature
dropped too low then it was warmed to its working temperature using a water bath. To ensure the
chocolate was in temper, the tip of a small spatula was dipped in the chocolate and left for a few
minutes at room temperature. Temper was achieved if after a few minutes the chocolate was
hard, glossy, smooth, and lacked streaks. The properly tempered chocolate was then moulded
and placed in the fridge for 20 min. A cheese grater with fine grating slots was used to shave the
chocolate into small seeds. The seed chocolate was then used with the Revolation 2 machine to
temper the chocolate. Chocolate was added to the assembled machine, melted and brought to
34.4°C. Once melted, the chocolate was cooled to the working temperature mentioned
previously. During this cooling period, seed chocolate was slowly added to the melted chocolate.
The amount of seed added was approximately 3-6% by weight of the total weight of chocolate. A plastic spatula was used to enhance mixing during this stage. When the working temperature was achieved the chocolate was checked as above to make certain it was in temper. The EC mix was then added to the tempered chocolate and the procedure continued as described above. All chocolate was moulded into a tablet that was 3.60 cm by 1.90 cm with a depth of 0.68 cm.

After storage at room temperature for one night the EtOH was evaporated from the HRC in an incubator at 40°C unless otherwise indicated.

3.2.3 Heat Resistance Measurements

Heat resistance of the chocolate was tested using a Texture Analyser (Stable Micro Systems Ltd, Surrey, UK) with a cylinder probe having a diameter of 1.80 cm. The testing mode was set displacement in compression with a displacement of 4 mm. Following removal of the EtOH samples were placed in an incubator at 40°C, unless otherwise indicated, for 2 h prior to testing. Samples were then individually transferred to the tester stage and placed with their centre directly below the cylindrical probe. The test was run and the force (N) at 2 mm displacement was recorded. This method was used to obtain a numerical measure of heat resistance for all samples tested.

3.2.4 Effect of EC Viscosity on Heat Resistance

HRC was made in the manner described above using compound milk or dark chocolate. EC of various viscosities was mixed with EtOH to give a 20% solution in the EtOH. EC used included 4, 10, 20, and 45 cP. The chocolate was prepared, dried, and tested for heat resistance at 40°C using the texture analyser as described above.

3.2.5 Measuring Ethanol Loss

Ethanol loss from the chocolate was measured during various evaporation treatments. HRC was prepared by adding 10% of a mix of 20% EC 45 cP in EtOH to compound milk chocolate. The chocolate therefore contained 8% EtOH. The chocolate was prepared as described above. Immediately following demoulding two pieces of chocolate were added to weighed tin containers and the initial total weight was measured and recorded. Three tins of chocolate were maintained in one of six drying environments: 20°C or 50°C with vacuum (13 kPa), 30°C, 30°C
wrapped, 40°C, or 50°C. Each piece of chocolate in the 30°C wrapped treatment was individually wrapped with a single layer of aluminum foil. The samples were periodically weighed. The weight percent of ethanol lost after time \( t \) in the drying treatment was calculated using equation 3.1:

\[
\text{Ethanol loss} = \frac{w_i - w_t}{w_i \times x} \times 100\%
\]  

where \( w_i \) is the initial weight of the chocolate, \( w_t \) is the weight of the chocolate at time \( t \), and \( x \) is the initial fraction of ethanol in the chocolate (approximately 0.08). This formula assumes that ethanol loss begins after the initial weighing of the chocolate and that all weight loss during the treatment is attributed to evaporation of ethanol.

### 3.2.6 Effect of Thickness on Ethanol Loss

HRC samples were prepared by adding 10\% of a mix of 20\% EC 45 cP in EtOH to compound milk chocolate. Cavities in the mould were filled to various depths with the chocolate then the chocolate was cooled and demoulded. The thickness of the chocolate was then measured at the approximate centre using a digital micrometer. Samples were then placed in tins and initial weights recorded. The sample was weighed again periodically throughout the evaporation period. Samples were held at 30°C or 40°C and one set was wrapped in foil. Ethanol loss was calculated using equation 3.1. The following first order kinetic equation (equation 3.2) was fit to the ethanol loss data using GraphPad Prism 5.0 software,

\[
y = y_{\text{max}} \cdot \left(1 - e^{-K \cdot t}\right)
\]

where \( y \) is ethanol loss, \( y_{\text{max}} \) is the maximum ethanol loss, \( K \) is the rate constant, and \( t \) is time. The rate constant and its standard error were plotted for the various thicknesses at each of the drying conditions. The thickness reported in the graph was the average and standard error of the samples from the three drying conditions.

### 3.2.7 Measuring Surface Lightness

The surface lightness of chocolate samples was measured pre- and post-EtOH evaporation to observe any changes attributed to this treatment. A HRC sample as well as an EtOH control and a plain compound milk chocolate control were tested. The HRC was made with compound milk
chocolate and 10% of a mix of 20% EC 45 cP in EtOH. The EtOH control was made with compound milk chocolate and 8% EtOH. The plain compound chocolate control was made with compound milk chocolate. The initial surface lightness of each sample top and bottom were measured using a HunterLab Colorimeter (Reston, VA). The chocolates were then placed in tins and subjected to the evaporation treatment for the amount of time required to remove all EtOH from the HRC determined from the EtOH loss experiment. The surface lightness was measured again once the chocolate was free of EtOH.

3.2.8 Oil migration

HRC samples were prepared with compound milk or compound dark chocolate, and a 20% mix of EC of various viscosities dissolved in EtOH. Control samples with the corresponding amount of EtOH but no EC, and plain compound chocolate controls were also prepared. EtOH was evaporated from the samples at 30°C for 9 days. The chocolates were then individually weighed, placed on weighed Whatman #4 filter papers, and incubated at 40°C for 10 days. The filter papers were then re-weighed and the oil loss was calculated using equation 3.3:

\[
\text{Oil loss} = \frac{\text{paper}_f - \text{paper}_i}{\text{chocolate}_i} \times 0.31 \times 100\%
\]

where paper\(_f\) and paper\(_i\) were the final and initial weights of the filter paper, chocolate\(_i\) was the initial weight of the chocolate, and 0.31 is the initial fraction of fat in the total chocolate.

3.2.9 Melting profile

The melting profiles of HRC made with compound milk or dark chocolate were observed using a Q2000 differential scanning calorimeter (DSC) (TA Instruments, New Castle DE). Plain chocolate controls, EtOH controls, and samples containing 2.17% EC 20 cP from a 20% in EtOH mix were analyzed. A portion from the interior centre of a tablet of the chocolate was used for analysis. Samples were placed in Alodined-Aluminum hermetically sealed pans. The samples were crystallized using the same procedure so that the observed melting curves would show any differences due to the added ingredients. The samples were then heated to 60°C for 2 h and then cooled at 5°C for 30 min and stored at 20°C for one week. The melting behavior was studied from a temperature of -20°C to 60°C at a rate of 5°C/min.
3.2.10 Efficacy of other solvents for manufacture of HRC

Solvents other than EtOH were also examined for their ability to be used in the HRC manufacturing process. Limonene and the more volatile ethyl acetate (EA) were used to dissolve EC and HRC was prepared in the conventional manner. The heat resistance of the HRC made with EA was also tested. Samples were prepared using 20% mixes of EC 10 cP in either EtOH or EA. The concentration of EC in the final samples was 2.17%. Control samples were also prepared with the corresponding concentration of solvent but without any EC.

3.2.11 Statistical analyses

All statistical analyses were done using GraphPad Prism 5.0 software. Data was compared to identify significant differences between samples using one-way ANOVA with Tukey post-test. The confidence interval was 95%; samples with different letters were found to have statistically significant differences of their means.

3.3 Results and Discussion

3.3.1 Heat Resistance

Figure 3.1 below shows the heat resistance measurements of samples made with 2.17% EC 20 cP from a 20% EC in EtOH mix in compound milk chocolate as determined by mechanical testing. Heat resistance was measured at various temperatures. It is clear that the chocolate with EC at 40°C is not nearly as hard as the control at 20°C. The control at 40°C was completely melted and flowable and showed no heat resistance. In contrast the sample with EC showed heat resistance at 40°C and even up to 80°C. A control chocolate which was made with an equivalent amount of EtOH but no EC also showed some heat resistance at 40°C. This is not yet fully understood however this heat resistance could be caused by small amounts of water added to the chocolate via absorption to the EtOH. The water can cause the surface of the sugar particles in the chocolate to dissolve and become sticky. The sugar particles can then stick to one another creating a sugar network which can resist deformation and trap liquid fat at elevated temperatures (Stortz & Marangoni, 2011). It is also speculated that the EtOH itself may play a role in the development of heat resistance since some of the phospholipids in soy lecithin are soluble in EtOH (Teberikler, Koseoglu, & Akgerman, 2001). Soy lecithin is the emulsifier commonly used in chocolate to coat the surface of the sugar particles to reduce the viscosity of
the chocolate melt (Beckett, 2000). If some of the phospholipids present in soy lecithin are removed from the surface of the sugar then perhaps this would increase the viscosity of the molten chocolate or allow for the sugar particles to interact with one another creating a network to resist deformation and trap liquid fat.

Figure 3.1 Hardness of control (no EC) and solvent substitution compound milk chocolate with 2.17% EC 20 cP from a 20% EC in EtOH mix tested using a mechanical tester

Figure 3.2 below shows the heat resistance of three types of chocolate with varying amounts of EC 22 cP. There was a general positive trend of increasing heat resistance with increasing amounts of EC. There are also clear differences in heat resistance between the different chocolate types. The milk and dark chocolates have similar fat contents but dark chocolate has much lower heat resistance. Furthermore, milk and white chocolate have similar heat resistance even though milk chocolate has 6% more fat than the white chocolate. Clearly the fat content of the chocolate is not the only contributing factor from the chocolate formulation that is affecting the heat resistance. It is necessary to better understand the mechanism of heat resistance in this chocolate to explain the differences observed between these chocolate formulations. It is thought that differences in sugar content may be a contributing factor for the differences observed. This graph also shows formulas using mixtures of either 20% EC in EtOH or 25% EC in EtOH. Chocolates made with a 20% EC in EtOH solution showed superior heat resistance compared to chocolate made with 25% EC in EtOH. Perhaps the EC in the 20% solution was better
solubilized which allowed for greater heat resistance. Alternatively, the presence of more EtOH in the samples made with the 20% solution may have been a factor in their greater heat resistance.

![Figure 3.2](image)

Figure 3.2 Hardness of samples made with chocolate and EC 22 cP at 40°C. Numbers in brackets refer to concentration of EC in the EtOH mix.

We observed that some formulas were difficult to mould due to their high viscosity. Such samples included the formulas with the two largest amounts EC for all types of chocolate made with 20% EC in EtOH and the formula with the largest amount of EC for all types of chocolate made with 25% EC in EtOH. A decrease in heat resistance was observed with additional EC added to the formula that was difficult to mould for milk chocolate. Clearly there was an upper limit for EC dispersability.

### 3.3.2 Effect of EC viscosity on heat resistance

The results in figure 3.3 show that there are significant differences in heat resistance of chocolate made with EC of various viscosities. However, there is no clear pattern indicating that one EC viscosity is superior to another. It is therefore thought that the size of the polymer, which is related to its viscosity, has little impact on the heat resistance of the compound chocolate. The data from all four EC viscosities was averaged to show how the heat resistance of the chocolate increases linearly with increases in concentration of the EC (figure 3.4). Similar observations were made with HRC made with compound dark chocolate (figure 3.5). In all cases chocolate containing EC was significantly harder than the control made with added EtOH but no EC.
Figure 3.3 Hardness of samples at 40°C made with increasing amounts of EC of varying viscosity from a 20% EC in EtOH mix in compound milk chocolate. Different letters indicate statistically significant differences within that group (p<0.05)

Figure 3.4 Hardness at 40°C of compound milk chocolate made with increasing amounts of a mix of 20% EC in EtOH. Each point represents the averages and standard deviations of samples made with EC 4, 10, 20, and 45 cP

3.3.3 Drying of HRC

The time to remove the EtOH from the HRC is an important parameter for manufacturers to know. Results in figure 3.6 indicate the storage conditions in descending order of time for ethanol loss was: 50°C with vacuum (13 kPa), 50°C, 40°C, 30°C, 30°C wrapped in aluminum foil, and 20°C with vacuum. The approximate drying times for HRC made with compound milk chocolate and 10% of a mix of 20% EC 45 cP in EtOH under the various storage conditions are
indicated in table 3.2. During sample preparation, an odor of ethanol was observed when the EC mix was added to the molten chocolate. This is evidence that when the time zero weight was taken the chocolate had already lost some unknown amount of ethanol and can explain why samples never appear to reach 100% ethanol loss.

Figure 3.5 Hardness at 40°C of compound dark chocolate made with a 20% mix of EC of various viscosities in EtOH. Different letters indicate statistically significant differences (p<0.05)

Figure 3.6 Ethanol loss from HRC during storage

Table 3.2 Approximate drying times for HRC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drying Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C Vacuum</td>
<td>12</td>
</tr>
<tr>
<td>50°C</td>
<td>30</td>
</tr>
<tr>
<td>40°C</td>
<td>90</td>
</tr>
<tr>
<td>30°C</td>
<td>210</td>
</tr>
<tr>
<td>30°C Wrapped</td>
<td>280</td>
</tr>
</tbody>
</table>
3.3.4 Effect of chocolate thickness on ethanol loss

The results in figure 3.7 indicate a negative correlation between rate of EtOH loss and thickness of HRC. This is expected since gas permeability is inversely proportional to the thickness of the material (Stookey, 2006). This trend was observed during three different drying treatments; 30°C wrapped, 30°C, and 40°C. This figure also shows that the rate of ethanol loss was highest at 40°C followed by 30°C and was lowest when stored at 30°C and wrapped in foil. Again, this was expected as gas permeability is directly proportional to the temperature (Stookey, 2006).

![Rate constant K with standard error for EtOH loss from HRC of increasing thickness.](image)

3.3.5 Effect of drying treatment on surface lightness

In terms of time to dry, HRC dried at 50°C under vacuum was the fastest. However, some surface defects were observed. Therefore, the change in surface lightness of the chocolate after the various heat treatments was measured. The lightness of a control compound chocolate was compared to that of HRC and a chocolate made with 8% EtOH (no EC). Results in figure 3.8 below indicate that drying HRC at 40 or 50°C resulted in a significant increase in lightness of the mold-contacting face compared to the initial control chocolate. The atmosphere-contacting face did not significantly increase in lightness compared to the initial measurement. However, the lightness was higher at 40 and 50°C than at 30°C. It is clear that to avoid surface defects the HRC should be dried at 30°C. Furthermore, the EtOH treated chocolate was not significantly
different from the HRC at any of the drying treatments indicating that perhaps the EtOH is responsible for some of the increase in surface lightness of the HRC.

![Graph showing surface lightness of dried HRC](image)

Figure 3.8 Surface lightness of dried HRC. (a) Mold-contacting face; (b) atmosphere-contacting face. CMC is compound milk chocolate, HRC is heat resistant chocolate, and EtOH is ethanol control chocolate

### 3.3.6 Oil migration

The results clearly indicate that oil loss at elevated temperatures is significantly reduced in the samples which contain EC (figure 3.9A). Interestingly, oil loss was also reduced in the EtOH controls (figure 3.9B). The EtOH controls were made by adding EtOH to compound milk chocolate at a concentration equivalent to what would be present in the HRC which contained both EC and EtOH. It is also clear that for the EtOH control samples, a greater amount of EtOH is required for a larger reduction in oil loss. Similarly to what was mentioned in section 2.3.1 it is thought that the EtOH may be removing emulsifiers from the surface of the sugar in these chocolates allowing for the sugar particles to contact one another and create a network which resists oil migration. Again, a second explanation could be the presence of very small amounts of water in the EtOH possibly by absorption from the atmosphere during the drying period which could help create a sugar network in the chocolate (Stortz & Marangoni 2011) and slow oil migration. The trend of decreasing oil loss with increasing EtOH was not observed when EC was also present in the chocolate. Further, it was observed in both compound milk and compound dark chocolates that EC of various viscosities showed similar amounts of oil loss and
this oil loss was also not significantly different than what was observed in the EtOH control (figure 3.10). One possible outlier was observed in the compound milk chocolate sample made with 2.17% EC 20 cP which showed slightly higher oil loss than expected. These results seem to indicate that the presence of EtOH in the samples is partly responsible for restricting oil loss at elevated temperatures. It is also clear by observing the samples with low EC concentrations compared to the EtOH controls that the EC is playing a role in preventing oil loss. For example, the sample with 1.04% EC 20 cP showed much less oil loss (1.3%) compared to the sample with an equivalent amount of EtOH (4%) which lost 5.3% of its total fat.

Figure 3.9 Oil loss after 10 days at 40°C from (A) HRC made with compound milk chocolate (CMC) and various concentrations of EC 20 cP and (B) control compound milk chocolates made with initial concentrations of EtOH equivalent to the initial concentration of EtOH in the samples with both EC and EtOH. EtOH was evaporated from all samples prior to oil migration testing.

3.3.7 Melting profile

The results from the DSC analyses of the melting profiles of control compound milk chocolate, compound milk chocolate with EtOH, and compound milk chocolate with 2.17% EC from a mix of EC in EtOH revealed differences between these treatments. Major differences were observed between the control sample and the samples which were made with EtOH which was evaporated prior to the DSC run. The control samples had peak melting temperatures 2°C and 1.5°C lower than the samples which contained EtOH for compound milk and dark chocolates respectively. The melting temperatures were still within the desirable range for chocolate which is from 20°C-
36°C (Aguilera & Lillford, 2008). It is believed that some of the minor polar components which may be present in the palm kernel oil of the chocolate such as sterols or triterpene alcohols (Goh, Choo, & Ong, 1985; Itoh, Tamura, & Matsumoto, 1973) may have been displaced from the centre of the chocolate upon evaporation of the EtOH due to their solubility in this solvent (Liu, 2011). The removal of these components likely affected the crystallization of the fat leading to the slightly higher melting point observed.

Figure 3.10 Oil loss after 10 days at 40°C from HRC made with 2.17% EC of various viscosities in (A) compound milk chocolate (CMC) or (B) compound dark chocolate (CDC)

3.3.8 Efficacy of other solvents for manufacture of HRC

It was found that EC was marginally soluble in limonene but the mixture was not dispersible in chocolate. EC was soluble in ethyl acetate and a mix of 20% EC 22 cP in ethyl acetate was used to prepare HRC. The samples were dried at 30 or 40°C; the drying curve is shown in figure 3.11. Ethyl acetate was removed from the HRC in less time than EtOH. However, as seen in figure 3.12 samples made with EC in ethyl acetate showed significantly reduced heat resistance at 40°C compared those made with EtOH. It was observed that the addition of EC to the system resulted in an increase in heat resistance compared to the controls that contained the solvent but no EC. This was observed in both compound milk and compound dark chocolate. These results indicated that although the use of ethyl acetate would shorten the drying time of HRC, EtOH provides much greater heat resistance. One explanation for the difference in heat resistance of samples made with EtOH compared to those with ethyl acetate is that the lecithin phospholipids which are soluble in EtOH are not soluble in ethyl acetate (Janek, 1987). This agrees with the idea that
removal of lecithin phospholipids from the surface of the sugar could play a role in developing heat resistance within this HRC.

![Figure 3.11 Comparing drying times for HRC made with EtOH or ethyl acetate (EA)](image)

Figure 3.11 Comparing drying times for HRC made with EtOH or ethyl acetate (EA)

![Figure 3.12 Heat resistance of (A) compound milk chocolate and (B) compound dark chocolate with EC 10 cP dissolved in EtOH or ethyl acetate (EA)](image)

Figure 3.12 Heat resistance of (A) compound milk chocolate and (B) compound dark chocolate with EC 10 cP dissolved in EtOH or ethyl acetate (EA)

### 3.4 Conclusions

Many efforts have been made to prepare a HRC for sale in tropical locations or during hot months of the year that is capable of withstanding temperatures above the melting point of cocoa butter. With the novel techniques and formulas described in this paper it is clear that addition of EC to chocolate can provide heat resistance. The heat resistance can be tailored by optimizing the ratio of EC to EtOH in the solution of EC, and the amount of EC added. Furthermore, oil
migration at elevated temperatures from the chocolate has been substantially reduced. EtOH has been identified as a solvent of choice for producing the HRC and several methods to remove the EtOH are possible. Future work should focus on understanding the mechanism by which heat resistance is induced in this system. Furthermore, the removal of EtOH should be optimized and an EtOH recovery step introduced.

References


CHAPTER 4

Molecular interactions of sucrose and ethylcellulose

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Abstract

Recent work has shown that sucrose crystals have the ability to interact with ethylcellulose (EC) and form a network within food materials that provided mechanical strength and increased stability. We recently reported on the ability of EC to impart heat resistance in chocolate.\textsuperscript{1} While this resistance is partly due to polymer gelation of the cocoa butter, recent evidence suggests that the sucrose present plays a major role in this effect. Here we show that EC is able to hydrogen bond with sucrose which allows for the creation of an oil-trapping network. Atomic scale molecular dynamics simulations and Fourier-transform infrared spectroscopy both showed the ability of EC to hydrogen bond with sucrose. These interactions were shown to be specific for crystalline particles with a glucose moiety having the hydroxyl group on carbon 2 free to hydrogen bond with the EC. Texture analysis and scanning electron microscopy showed the ability of EC and sucrose to form a network within an oil medium which resisted deformation. It was also shown that lecithin at the surface of the sucrose impedes EC-sucrose interactions. These results have lead to an understanding of the mechanism of heat resistance in EC solvent substitution chocolate and will be useful in developing new food products containing EC as a sugar network enhancing functional ingredient.

4.1 Introduction

It has previously been shown that addition of ethylcellulose (EC) to conventional chocolate using the “solvent substitution” method has resulted in production of a chocolate with substantial heat resistance.\textsuperscript{1} A heat resistant chocolate is one that can resist melting or deformation at temperatures above 34°C, the normal melting temperature of chocolate.\textsuperscript{2} Many methods have been developed to produce heat resistant chocolate.\textsuperscript{3} The addition of EC to induce heat resistance in chocolate represents a novel technique and the mechanism of heat resistance will be explored.
Although the patent literature reviewed did not explain a mechanism responsible for heat resistance in chocolate, several important observations have been made. In our analysis, it became obvious that many of the methods developed ultimately lead to the creation of a sugar network in the chocolate using a variety of techniques. The secondary sugar network allowed the chocolate to hold its shape when the primary fat crystal network has melted. This could be done by wetting the surface of the sucrose crystals with small amounts of water so that it becomes “sticky” and would aggregate with other solid particles in the chocolate. Large amounts of water could also be used to completely dissolve the sucrose and eventually the water was evaporated to leave behind a recrystallized sucrose network. Furthermore, other methods were developed which prevented the sucrose crystals in the chocolate from being fully coated with fat, leaving some surfaces of the crystals available to stick to one another and form a network. All of these methods utilized the sucrose in the chocolate to form a secondary network.

In the EC solvent substitution (SS) method, 20% EC was dissolved in absolute ethanol (EtOH) and then added to a molten, conventional chocolate. The resulting chocolate with 2.17% EC had a hardness of 18 N at 40°C. It was hypothesized that the EC was able to interact with the sucrose and create a network within the chocolate that lead to the observed heat resistance. This hypothesis will be explored.

EC is a linear polymer of β-1,4 linked D-glucose units with some of the hydroxyl groups substituted with ethoxyl groups (Figure 4.1). There are three hydroxyl groups on each monomer (excluding terminal monomers) that are available for ethoxylation. The degree of substitution is controlled during manufacture; commercially produced EC that is soluble in organic solvents has a degree of substitution of 2.3-2.6 out of a possible three. The EC polymers used in this study were specified as having an ethoxyl content of 48-49.5% which corresponds to a degree of substitution of 2.47-2.58. Previous studies have shown that the distribution of ethoxyl groups on the monomers is not random. The group showing lowest reactivity is R3. This is thought to be a consequence of intramolecular bonding between the hydrogen of the hydroxyl group at R3 and the ring-oxygen atom in adjacent monomers. Interestingly, it was found that the relative reaction rate of ethoxylation at R3 increases when R2 is ethoxylated which is thought to be due to disruption of the previously mentioned intramolecular hydrogen bond. The hydroxyl group at R6 was found to be most reactive. With three hydroxyl groups available for ethoxylation
there are eight possible monomers in an EC polymer. The concentrations of each type of monomer were not available for the EC polymers used. Therefore, these values were found in the literature and used as close approximations for what would be present experimentally.

Figure 4.1 Structure of ethylcellulose with carbon and R-group numbering system

It is not obvious from the literature whether interactions can exist between EC and sucrose. Ibrahim et. al.\textsuperscript{11} coated sucrose crystals with EC by first dissolving the EC in EtOH and thereafter evaporating the EtOH. The EC coating was used to reduce the solubility of sucrose in an aqueous system for the purpose of understanding the effect of excipient solubility on the dissolution of a drug prone to agglomeration. Scanning electron photomicrographs showed that the EC covered the sucrose and dissolution of the covered substance only occurred at holes in the coating where the aqueous phase was able to penetrate.\textsuperscript{11} Sucrose was also mentioned as a low molecular weight additive which could be used to increase the permeability of an EC coating for controlled-release of pharmaceuticals.\textsuperscript{12} Considering the structure of EC and sucrose (Figure 4.2A) it is thought that hydrogen bonding may occur between polar groups on EC and sucrose, such as unsubstituted hydroxyl groups on the EC chain, and free hydroxyl groups on the sucrose crystal.

It is also important to consider the possibility of an interaction between EC and the lecithin phospholipids present at the surface of sucrose in conventional chocolate. A specific interaction between soybean phospholipids and EC has not been shown. However, interactions between EC and dialkyl phthalates\textsuperscript{13}, propylene glycol dicaprylate/dicaprate\textsuperscript{14}, and nifedipine\textsuperscript{15} have been
found. All of these molecules have similar structure to phosphatidylcholine (PC) (Figure 4.2B), an abundant phospholipid in soybean lecithin. It is thought that since the hydroxyl groups on EC are slightly polarized they can hydrogen bond to the slightly polarized carbonyl groups at the ester linkage in PC. In contrast, no interactions were found between the carbonyl groups on triglycerides and EC. If an interaction between EC and PC exists then perhaps the PC can act as a bridge between EC and sucrose during network formation.

![Figure 4.2 Structure of (A) sucrose with carbon numbering system, (B) phosphatidylcholine, and (C) glucose](image)

**4.2 Materials and methods**

**4.2.1 Atomic scale molecular dynamics (MD) simulations**

Atomic scale molecular dynamics simulations were performed to explore the hypothesis that EC and sucrose can hydrogen bond to one another. The interactions between a representative EC molecule and a PC, specifically 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) (Figure 4.3A), and POPC and sucrose were also explored. Initially, the polymer was only 30 monomer units in length due to computing limitations. The proportions of each of the eight possible EC monomers were chosen based on the work by D’Ambra et al. and are shown in
Table 4.1. D’Ambra et al. used a commercially available EC with a degree of substitution of 2.66. This degree of substitution was slightly higher than the degree of substitution for the EC polymers used experimentally which was 2.47-2.58. Nevertheless, the proportions found by D’Ambra et al. were thought to give a relatively good estimate of the monomer proportions present in the EC used. This molecule will be hereafter referred to as “EC30”. The sequence of monomers in EC30 (Figure 4.3B) was chosen deliberately to have three fully ethoxylated monomers between each doubly ethoxylated monomer since the presence of the former is much greater than the latter. A representative sucrose crystal (Figure 4.3C) was built by adding 41 sucrose molecules to a 3 nm$^2$ unit cell and running an unrestrained simulation for 5 ns to allow the sucrose molecules to orient themselves in a crystal structure, maximizing hydrogen bonds between their hydroxyl groups.

Simulations were performed using GROMACS 4.5.5 software suite.\textsuperscript{18-21} Sucrose and EC were built using the 53A6\textsuperscript{22} subset of the GROMOS force field, with parameters adapted from the 45A4\textsuperscript{23} subset for pure carbohydrate systems and subsequent re-optimized parameters from the 56A\textsubscript{carbo}\textsuperscript{24} force field. Parameters for the ethoxy groups were adapted from parameters developed for ethoxyethane from the Automated Topology Builder web server.\textsuperscript{25} POPC parameters were taken directly from work previously done by Tieleman et. al.,\textsuperscript{26} with parameters for triolein adapted from the acyl chains of POPC. Periodic boundary conditions were used for all simulations. Short-range electrostatics were cut off at 0.9 Å and long-range electrostatic interactions were calculated using the particle-mesh Ewald method. To calculate van der Waals interactions, a twin-range scheme was employed (0.9/1.4 Å). A 2 fs integration time step was used with neighbour searching performed every 5 ps. The Berendsen weak coupling\textsuperscript{27} method was used to couple temperature and pressure using the velocity rescale\textsuperscript{28} thermostat ($\tau_t = 0.1$) and Parrinello-Rahman\textsuperscript{29} barostat ($\tau_p = 0.5$) respectively at 307.15 K and 1 bar. Avogadro 1.0.3\textsuperscript{30} was used to build the initial structures for both the EC and triolein molecules. Hydrogen bonding was analysed using a program which identifies these bonds based on two geometrical criteria:

$$r \leq r_{HB} = 0.35 nm \quad 4.1$$
$$\alpha \leq \alpha_{HB} = 30^\circ \quad 4.2$$

where $r$ is distance between donor and acceptor and $\alpha$ is the angle between hydrogen donor and acceptor.\textsuperscript{31} These values are based on observations of hydrogen bonding found in water and are widely used in analysis of molecular simulations.\textsuperscript{31}
The system was reevaluated and several changes were made prior to moving forward with subsequent simulations. Two new EC molecules of 62 monomers length were built with proportions of monomers based on the work by Rosell\textsuperscript{7} and are given in Table 4.2. Rosell analyzed EC with a degree of substitution of 2.64, similar to D’Ambra et al. and still higher than the DS of the EC used experimentally. However, Rosell found slightly different proportions of monomers than D’Ambra. The differences observed were likely due to the different methods used which have been described in detail elsewhere\textsuperscript{7,17}. The proportions determined by Rosell and use of a longer molecule permitted the inclusion of a greater number of hydroxyl groups within the EC molecule allowing for a greater number of possible hydrogen bonds to be observed. The distribution of each of the monomers in an EC polymer is unknown therefore two
distinctly different distributions were chosen to be studied. The first new EC molecule had two fully ethoxylated monomers between each of the hydroxylated monomers and will be henceforth denoted as “ordered” polymer. The second polymer had blocks of five hydroxylated monomers between ten fully ethoxylated monomers and will be denoted as “co-block” polymer. The large blocks of hydroxylated monomers may mimic a crystalline region along the EC that was unable to be ethoxylated during manufacture.

Four new systems were studied utilizing the ordered and co-block EC, POPC, the sucrose crystal, and triolein as a representative triglyceride. The first system included the EC molecule solvated in triolein; the second system had sucrose crystals placed next to the EC molecule and this was solvated in triolein; the third system had the sucrose crystals coated in POPC next to EC molecules and this was solvated in triolein; the fourth system had EC solvated in triolein with sucrose crystals randomly placed within the simulation space (at a further distance from EC than system two). The simulations were performed and analyzed using the same settings as described in above except that the temperature was chosen as 40°C to mimic the temperature used when performing heat resistance tests.

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</tr>
</tbody>
</table>

Total = 0.73

### 4.2.2 Hardness tests of model systems

#### 4.2.2.1 EC interacting with sucrose

Model chocolate systems based on the ingredients in solvent substitution (SS) chocolate were prepared using the following ingredients: granulated sucrose (Red Path, Toronto ON), EC 10 cP (Dow Chemicals, Midland, MI), palm kernel oil (PKO) (Nealander’s International Inc.,
Mississauga, ON), soybean lecithin (Grain Process Enterprises Ltd. Scarborough, ON), glycerol monooleate (GMO) (Hallstar, Chicago IL), sorbitan monooleate (SMO) (Sigma Aldrich, St. Louis, MO), Alphadim 90 SBK glycerol monostearate (GMS) (Caravan Ingredients, Lenexa, KS), Grinsted sorbitan monostearate (SMS) (Danisco, Scarborough, ON), and absolute ethanol (EtOH) (Commercial Alcohols, Brampton, ON).

Two methods were used for preparation of these samples: the solvent substitution method and the heat method. Granulated sucrose was chopped in a blender prior to use to reduce the particle size. For the SS method the PKO was melted and the sucrose, when used, was added and thoroughly mixed. If sucrose was not added then the PKO was left at room temperature to begin crystallisation. Once crystallisation began the PKO was stirred occasionally until it was partially crystalline but still flowable. EC 10 cP from a 20% EC in EtOH mix was then added to the PKO or PKO and sucrose mixture and stirred for 1 min using an overhead stirrer at 200 rpm with a paddle impeller. The sample was then moulded, cooled at 5°C for 20-30 min, and demoulded by rapping the mould on a counter. The mould produced samples in tablet form with dimensions of 3.60 cm (l) x 1.90 cm (w) x 0.68 cm (d). The samples were left overnight and then placed in an incubator at 30°C for sufficient time to evaporate the EtOH.

Heat method samples were prepared by dissolving the EC in the PKO, with surfactant if used, in a beaker with heating (up to around 140°C) and stirring. It was required to heat the sample to 140°C, the glass transition temperature of EC, to fully dissolve the EC. For samples containing sucrose the EC and PKO was poured into the sucrose sample once cooled slightly (around 110°C). The sucrose was kept in a stainless steel 250 mL beaker that was held at 60°C using a dry bath with a custom fitted steel sample holder. This ensured the temperature of the sample remained elevated during stirring and thereby minimized the chance that the EC would set prior to the end of mixing. The sample was stirred for 1 min at 200 rpm using an overhead stirrer with a paddle impeller. The samples were then moulded, cooled, and demoulded as in the SS procedure.

Some heat method samples were also prepared with lecithin. The amount of lecithin added was calculated based on maintaining a ratio of lecithin to solids similar to what would be found in a chocolate made with the typical amount of 0.3% lecithin and 70% solids-non-fat. During
preparation of these samples the lecithin was added to the molten EC in PKO gel once slightly cooled, just prior to mixing with the sucrose. The samples were then moulded, cooled, and demoulded as above.

Samples with sucrose were formulated to contain a final concentration of 2.17% EC. Sucrose-free controls were formulated based on the assumption that all of the EC present would be in the fat phase and not interacting with the sucrose. Therefore, these controls contained the same ratio of EC:PKO as the equivalent sample with sucrose.

All samples were tested for heat resistance using the method developed by Stortz and Marangoni. A texture analyser (Stable Micro Systems Ltd., Surrey, UK) was used to perform a set displacement in compression test on a sample that had been incubated at 40°C for 2 h. The cylindrical probe with 1.80 cm diameter was lowered into the sample 4 mm and the force at 2 mm displacement was recorded as the hardness of the sample.

4.2.2.2 EC interaction with various particles

The interaction between EC and various particles other than sucrose was studied to better understand the specificity of the interaction. Various particles were chosen to be studied based on several factors. Glucose is a monosaccharide that makes up half of the sucrose molecule and therefore it has very similar properties and structure as sucrose. Anhydrous glucose was obtained from Thermo Fisher Scientific (Ottawa, ON). Native starch is made up of amylose and amylopectin which are polymers of glucose joined by α(1-4) glycosidic bonds. Amylopectin contains many branches joined by α(1-6) bonds. Native starch is also semi-crystalline. Pre-gelatinized starch has undergone a process of cooking the starch in water to dissolve the starch granule which ultimately leads to a loss of crystallinity. Native and pre-gelatinized potato starch products called Novation 1900 and Novation 6600 respectively were obtained from Ingredion (Brampton, ON). Cellulose is chemically similar to starch but is a linear polymer of β(1-4) linked glucose units. Microcrystalline cellulose (MCC) is a purified fraction of cellulose and as the name suggests it is highly crystalline. MCC was obtained in the form of spherical pellets called Cellets from Pharmatrans Sanaq AG (Basel, Switzerland). MCC in the form of fibers was obtained from Thermo Fisher Scientific (Ottawa, ON).
Rice bran wax (RBW) was chosen as a particle that is not similar to sucrose. Waxes are formed by the esterification of a long-chain aliphatic carboxylic acid with a long chain aliphatic alcohol. They are hydrophobic and similar to the vegetable oils that are traditionally used to form organogels with EC but due to their very long aliphatic chains and ester linkages they have much fewer polar functional groups. It is thought that there would be very few interactions between EC and waxes and the wax could be thought of as an inert particle. RBW was obtained from Koster Keunen, LLC (Watertown, CT). Other inert particles were also tested. Soda-lime silica glass beads were chosen as a non-crystalline inert particle and diamond dust was used as a crystalline inert particle. Glass beads were obtained from Potters Industries LLC (Valley Forge, PA) and diamond dust was obtained from Diamond Technologies Co., Ltd (Bangkok, Thailand).

The particles used were chosen to have a similar size to the sucrose used. For this experiment superfine sugar from Lantic Inc. (Montreal, QC) was used. Some of the materials required pretreatments to obtain a suitable particle size. The RBW pellets were chopped using a food processor and sieved to achieve a smaller particle size. Furthermore, potato starch was chosen because the native potato starch granule is one of the largest with diameters up to 110 µm. However, it was not possible to obtain a commercial native potato starch that had not been milled to a fine particle size. To overcome this, finely ground native potato starch was wetted with cold water, spread onto cooking sheets and placed in the oven at 80°C. After drying the starch was broken up into small pieces and crushed in a blender for further particle size reduction. The particles were then sieved through two sieves of 70 and 170 mesh size to obtain the middle fraction with a particle size of 89-211 µm.

The sizes of all of the particles were measured using a microscopic technique. Particles were deposited on glass microscope slides and micrographs were obtained under visible light using an Olympus BX60 light microscope with an attached Olympus DP71 camera (Olympus Canada Inc., Richmond Hill, ON) used to obtain digital images. Characterization of particle size was performed using the "count/measure" function built into the ImagePro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD) which automatically determines the boundaries of each particle and then measures the mean particle diameter. A minimum of three particles were measured on each slide and seven slides were characterized for each particle type.
Samples were prepared using the solvent substitution method explained above. Formulas were calculated to produce samples with an equal volume fraction of each type of particle. Since the particle size and volume fraction for each type of particle was relatively similar, the 2.17% EC in each sample would have a similar surface area to interact with for each of the particles. Literature values for the density were used to calculate the mass of the particles to add to each sample to obtain the correct volume fraction. The density of RBW was easily measured using a pycnometer and a scale and this value was used for the calculations.

All of the samples were tested for hardness at 40°C using the texture analyzer method already described. One modification to the analysis was made; the area under the curve (AUC) from 0-3 mm compression was used as a measure of hardness instead of the force at 2 mm displacement. This was necessary because the various samples had widely different properties; for example some of the samples would fracture after 2 mm displacement. Many measurements were analyzed and showed very similar trends however the data was best represented using the AUC.

4.2.3 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to observe if chemical bonding occurs between EC and sucrose. Two sample preparation and analytical techniques were used. The first method followed a similar procedure to the SS method discussed previously. A solution of 20% EC 10 cP dissolved in absolute EtOH was prepared. Granulated sucrose was chopped in a blender and then the particle size was further reduced by grinding in a mortar and pestle. The EC was then mixed at various proportions with the sucrose. The mixture was occasionally mixed as the EtOH evaporated and spread to avoid producing clumps with EtOH filled centers. Once seemingly dry the mixture was then placed in an oven at 80°C for 1 h to evaporate any residual EtOH. The sample, which was quite chunky at this point, was then ground to a fine powder using a mortar and pestle. The sample was again incubated at 80°C for 1 h to evaporate any remaining solvent. After heating, the sample was placed in a desiccator to cool. Finally, the sample was capped and stored in the desiccator. Samples were prepared to produce mixes of 16, 33 or 50% EC on sucrose once dried.

Appropriate controls were also prepared. Control samples were prepared using the same procedures but did not contain EC. For example, the control for the 16% EC from EtOH sample
was made by mixing sucrose with the same amount of EtOH as was present in the EC in EtOH sample. The EtOH was then evaporated, and the sample heated, ground, and heated again. Other controls included EC 10 cP powder and sucrose with no treatment.

A Thermo Fisher Scientific Nicolet 6700 FTIR spectrometer (Waltham, MA) with deuterated triglycine sulfate (DTGS) detector was used for analysis of the samples. Transmission mode was used for these powder-type samples. The spectra were collected as KBr pellets at 64 scans and had a resolution of 2 cm$^{-1}$. Each sample was analyzed in triplicate.

The second method of sample preparation mimicked the heat method. EC 10 cP was dissolved in canola oil by heating to 140°C with stirring. Once fully dissolved the solution was mixed briefly by hand with finely ground sucrose. The sample was then cooled and stored in a desiccator. Samples were prepared with either 2 or 5% EC. Control samples contained heated oil and sucrose without any EC.

The heat method samples were analyzed using the same spectrometer and the attenuated total reflectance (ATR) sampling technique. The samples were spread onto a zinc selenide (ZnSe) crystal sample holder. The spectra were collected from 64 scans with a resolution of 2 cm$^{-1}$. Each sample was analyzed in triplicate.

Samples were also prepared with the addition of lecithin phospholipids. A crude extract of soybean phospholipids containing 14-23% phosphatidylcholine (PC) was obtained from Sigma-Aldrich (Saint Louis, MO). The PC was dissolved in chloroform to produce a 1% solution which allowed for the PC to be distributed onto the surface of the sucrose. The amount of PC added was representative of what is present in a typical chocolate and was calculated as follows: it was assumed that chocolate contains 50% sucrose and up to 0.5% lecithin\textsuperscript{37} of which approximately 56% is phospholipids\textsuperscript{38}. This gives an approximately phospholipid content of 0.28%.

Considering a system of only sucrose and phospholipids and ignoring the other components of the chocolate the sucrose should be mixed with 0.6% phospholipids by weight. The PC in chloroform was added to finely ground sucrose and mixed using an overhead mixer. The chloroform was evaporated and a fine powder of PC coated sucrose was produced. This coated sucrose was then used to produce heat method samples and controls using the same procedures as outlined above. A control was also prepared with sucrose treated with chloroform but no PC.
Spectra were analyzed using Thermo Scientific OMNIC Spectra Software (Thermo Electron Scientific Instruments Corporation, Madison WI). Minimal processing techniques were used on the spectra to preserve the experimentally observed results. All spectra were first ratioed to their respective background spectra. For pellet type samples the background was a pellet of KBr with no sample present. For ATR samples the background was the ZnSe crystal with no sample present. Peak positions were then identified for samples and their respective EC-free controls with particular focus on peaks for sucrose that have been identified in the literature. The peak position for the sample was then subtracted from the average peak position for the EC-free control to give a value for the peak shift. Significant peak shifts were recorded and compared to known peak positions for sucrose.

4.2.4 Scanning electron microscopy (SEM)

SEM was used to visualize the sucrose-EC network that was thought to be present within HRC. Samples were prepared using the SS method and included: control compound milk chocolate (Bulk Barn, Richmond Hill, ON), control with 8% EtOH, and compound milk chocolate with 2.17% EC 10 cP from a 20% solution in EtOH. The EtOH was evaporated in an incubator at 30°C for 9 days. All samples were then placed in a hexane bath for 48 h to remove all of the fat and leave behind the network of solid particles. The samples were suspended on a mesh platform about one third of the way from the top of a 400 mL glass beaker. A magnetic stir bar was placed below the mesh. The beaker was filled with approximately 300 mL of hexane and sealed with aluminum foil to prevent evaporation of the hexane. The beaker was then placed on a stir plate and set to 250 rpm. After two days of extraction the sample was removed from the beaker using tweezers and stored at room temperature in a covered dish until further use. The samples were cut with a blade but ended up fracturing cleanly to give a good cross section of the interior of the chocolates. A small piece of the chocolate was then mounted using double sided tape onto a sample holder. The sample holder was then placed in an Emitech K550 Sputter Coater (Quorum Technologies Ltd., East Grindstead, UK) set at 20 mA for 2.5 min to give less than 20 nm of gold coating. Following sputter coating samples were placed in the S-570 SEM (Hitachi High-Technologies, Tokyo, Japan). Images were obtained at various magnifications and at locations across the entire sample plane using Quartz PCI software (Quartz Imaging Corporation, Vancouver, BC) for image capture.
4.3 Results and discussion

4.3.1 Atomic scale MD simulations

The first set of simulation results using EC30 showed that hydrogen bonding between the ring oxygen of EC and the hydroxyl hydrogen of sucrose is possible. EC simulations containing multiple EC molecules have also shown the capacity for both intra- and intermolecular hydrogen bonding in which R-group hydroxyls interact with a nearby ring oxygen atom. This is to be expected as an empirical understanding of EC tells us that intermolecular hydrogen bonding is required for gel formation. The formation of hydrogen bonds between hydroxylated R-groups of EC and the ether oxygen on either the sn-1 or sn-2 tails of POPC were also observed. Similarly, simulations have shown hydrogen bonds between sucrose hydroxyl groups and POPC ether groups.

The second set of simulations using ordered and co-block EC were evaluated. System one which consisted of only EC in a sea of triolein was analyzed for the root mean square displacement (RMSD) of the hydroxyl R-group hydrogen atoms as a function of time to examine the instability of the polar hydroxyl groups of EC in a mostly hydrophobic environment. Results showed that for both the ordered and co-block EC polymers there was an increased RMSD as a function of time (Figure 4.4). The hydroxyl groups increasingly move or oscillate as they reorient themselves to minimize interactions with the hydrophobic tails of triolein. It was also found that for the ordered and co-block polymers there were an average of 1.25 and 1.58 hydrogen bonds between the EC polymer and the ester groups on triolein respectively (Figures 4.5A and B).

![Figure 4.4 Root mean square displacement (RMSD) of hydroxyl hydrogens on (A) ordered and (B) co-block EC polymers in triolein (simulation one)](image)
In the second simulation with EC next to sucrose surrounded by triolein it was found that both EC polymer types were able to hydrogen bond with sucrose (Figures 4.5C and D). This is the same result as was seen with EC30. It was observed that the co-block polymer had a delay between the start of the simulation and when it reached a relatively unchanging average number of hydrogen bonds a point not observed in the simulation with the ordered polymer. This can be attributed to twisting or shifting of the co-block polymer caused by the hydrophilic blocks of hydroxylated monomers wriggling to maximize polar contacts with sucrose and minimizing non-polar contacts. The average number of hydrogen bonds with sucrose for the ordered and co-block polymers were 4.88 and 4.66 respectively.

![Figure 4.5 Number of hydrogen bonds formed between: (A) ordered or (B) co-block EC and triolein (simulation one); and (C) ordered or (D) co-block EC and sucrose surrounded by triolein (simulation 2)](image)

The third simulation had the sucrose crystals covered in POPC next to EC and surrounded by triolein. Both sucrose and EC were able to form hydrogen bonds with POPC. This mimics the result found for EC30. Unlike the simulation with EC30 however, no hydrogen bonds were observed between EC and sucrose due to a shielding effect of the POPC which kept the EC at a distance too far from the sucrose for hydrogen bonds to form. The programs used to analyze the simulations require a distance between donor and acceptor of less than or equal to 0.35 nm. It
was found that the average distance between EC donors and acceptors was around 4.6 and 3.9 nm for the ordered and co-block EC polymers respectively (Figures 4.6A and B). Again, hydrogen bonds were observed between both EC polymer types and triolein similar to the previous simulation scenarios.

The final simulation had the EC solvated in triolein with sucrose crystals randomly placed within the simulation space. In this scenario there were no hydrogen bonds observed between either the ordered or co-block EC polymers and sucrose over the entire simulation. This was due to a large separation distance between the EC and sucrose with little attractive force to move the molecules close enough together for hydrogen bond formation (Figure 4.6C). The results for co-block EC were almost identical and are therefore not shown.

![Image of graphs showing distance vs time for EC donors and acceptors in simulation scenarios.]

Figure 4.6 Average distances between EC donor and acceptors for: (A) ordered or (B) co-block EC in simulation three; and (C) ordered EC in simulation four

Overall the simulations have been successful in showing that under appropriate conditions hydrogen bonds can form between EC of various configurations and a sucrose crystal, POPC, and triolein. Further insight has also been gained into hydrogen bonding between sucrose and POPC and inter- and intra-molecular bonding of EC with EC. It has also been clearly shown that EC and sucrose surfaces must be relatively close together for hydrogen bonding to occur. Hydrogen bonding is effectively impossible between EC and sucrose when the sucrose is thoroughly surrounded by a layer of POPC. This is indicative that in a conventional chocolate if the sucrose is surrounded by POPC, other phospholipids, or perhaps any emulsifier, then the sucrose would be unable to hydrogen bond with EC and this likely negatively impacts the heat resistance of the chocolate.
4.3.2 Hardness tests of model systems

4.3.2.1 EC interacting with sucrose

The hardness of samples made with 2.17% EC and increasing concentrations of sucrose prepared using the solvent substitution method are shown in Figure 4.7A. Some samples were too soft to test and were therefore not included in the results. Control samples made with only EC in PKO, or sucrose with added EtOH in PKO showed very little hardness at 40°C. However, samples made with EC and sucrose resisted deformation and displayed an increasing hardness with increasing sucrose concentration. If only the sucrose and fat in a HRC are accounted for then the proportion of sucrose would be around 63%. The value of hardness at this concentration was around 20 N which is very similar to the value obtained for a HRC made with the same concentration of EC (around 18 N). The exponential increase in hardness with sucrose concentration observed is indicative of an interaction between EC and sucrose.

Samples were also prepared using the heat method and similar results were observed (Figure 4.7B). Both the EC and sucrose controls were much softer than the sample with both sucrose and EC. Once again an exponential increase in hardness with increase in concentration of sucrose was observed. Samples made via the solvent substitution method with EtOH tended to be approximately 3 times harder than those made with the same amount of sucrose via the heat method. This is thought to be due to a combination of better dissolution of the EC in EtOH compared to in the oil phase and the slight solubility of sucrose in EtOH. Better dissolution of the EC would lead to a more extended polymer which may be better able to interact with the sucrose creating a stronger network. Furthermore, the solubility of sucrose in EtOH is 0.7 mg/g solution at 37°C. The small portion of the sucrose that is dissolved in EtOH may act like glue between sucrose particles causing them to stick to one another and the glue may harden once the EtOH evaporates. This would also add to the strength of the EC-sucrose network within the sample and lead to a greater hardness at 40°C compared to the heat method samples.

Heat method samples were also prepared with lecithin and the results are also shown in Figure 4.7B. It is clear that the addition of lecithin greatly decreases the hardness of the samples with EC. This is likely due to the shielding effect caused by the boundary layer of lecithin on the sugar surface. The EC was not able to get close enough to the sucrose to interact. This was predicted by atomic scale molecular dynamics simulations. The lecithin also reduced the
hardness of the control with no EC. This is expected since lecithin is known to reduce the viscosity of chocolate by aiding in the flow of fat and sucrose particles past one another.\(^{37}\)

Figure 4.7 Hardness of model chocolate samples at 40°C made using the (A) solvent substitution and (B) heat methods

Samples were prepared via the heat method with various ratios of GMO:EC to observe the impact of this surfactant on the hardness of the system. It has previously been shown that addition of surfactants to EC oleogels results in increases in gel strength.\(^{42}\) Figure 4.8A shows that there were no significant differences (\(p < 0.05\)) in hardness for the various ratios of GMO:EC in this system except for at 70.7% sugar. At this concentration the expected result was that the higher the amounts of surfactant present, the greater the hardness of the system.
Similarly, samples were prepared with various types of surfactants at a ratio of 1:3 with EC and results are shown in Figure 4.8B. It was found that samples made with GMS were somewhat harder than the other samples. This was likely due to the creation of a secondary GMS network within the sample that was able to resist deformation because it was solid at 40°C. Generally, samples made with surfactant were harder than those without surfactant. The surfactant can plasticize the EC allowing the polymer to be more extended in the oil which would help it to better interact with the surface of the sucrose. Generally, various surfactants produce various degrees of EC plasticization based on characteristics such as the headgroup size. Though there may have been different degrees of plasticization of the EC based on surfactant ratios or types, this did not lead to any significant differences in hardness of the samples. These results indicate that the strength of the EC gel had little impact on the heat resistance observed. Furthermore, since the sucrose-free controls showed very little heat resistance it can be concluded that organogelation contributes minimally to heat resistance.
4.3.2.2 EC interaction with various particles

Micrographs of the particles used in this study are shown in Figure 4.9 and show the differences in size and morphology of the particles. The average particle diameter and values for density are reported in Table 4.3. The particle sizes range from an average of 140-534 µm. The sucrose and glucose samples had almost identical particle sizes and were clearly individual crystals with a distinct crystalline morphology. Both starch samples, the rice bran wax (RBW) and diamond samples were more randomly shaped with rough edges. The microcrystalline cellulose (MCC) pellets and glass beads were mostly spherical in shape. Finally, the MCC fibres were not easily viewed individually due to a large amount of clumping however there were some individual...
fibres viewed which are only a few micrometers thick but several tens of micrometers in length. This fibrous morphology was distinctly different from all others.

Figure 4.9 Light micrographs of various particles: (A) sucrose; (B) glucose; (C) native starch; (D) pre-gelatinized starch; (E) MCC pellets; (F) MCC fibers; (G) RBW; (H) glass beads; and (I) diamond. RBW and diamond are shown at a higher magnification and have a scale bar measuring 500 µm while all others have a scale bar of 200 µm.

The results in Figure 4.10 show the area under the displacement in compression curve for the various particles at increasing concentrations. Particles which were able to interact with the EC will show an increase in the area under the curve (AUC) as the concentration of the particles increases. This is because as the number of particles increases there are more opportunities for interaction with EC. Control samples with no EC indicate the proportion of the area that can be accounted for by simply creating a more close packed system. Clearly, for most samples this control value is quite low and more importantly, significantly lower than the area for the EC-containing sample. One exception is for the MCC fibers. During preparation of this sample it
was clear that there was hardly enough fat or EC in the system to sufficiently coat the surfaces of the MCC fibers. This was probably due to the fact that this sample had such a different morphology than any of the other samples. The long, thin fibers had a very large surface area compared to the other sample morphologies. Since this huge difference in surface area clearly had a large effect on the results, MCC fibers will not be compared to the other samples in the rest of this discussion.

Table 4.3 Particle densities and average diameters with standard deviations

<table>
<thead>
<tr>
<th>Particle</th>
<th>Density (g/mL)</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>1.59</td>
<td>271 ± 80</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.54</td>
<td>273 ± 78</td>
</tr>
<tr>
<td>Native starch</td>
<td>1.54</td>
<td>193 ± 51</td>
</tr>
<tr>
<td>Pre-gelatinized starch</td>
<td>1.54</td>
<td>369 ± 138</td>
</tr>
<tr>
<td>MCC pellets</td>
<td>1.46</td>
<td>169 ± 40</td>
</tr>
<tr>
<td>MCC fibers</td>
<td>1.46</td>
<td>140 ± 64</td>
</tr>
<tr>
<td>RBW</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>534 ± 270</td>
</tr>
<tr>
<td>Glass beads</td>
<td>2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>163 ± 39</td>
</tr>
<tr>
<td>Diamond</td>
<td>3.52&lt;sup&gt;48&lt;/sup&gt;</td>
<td>212 ± 59</td>
</tr>
</tbody>
</table>

<sup>a</sup>measured  
<sup>b</sup>provided by the manufacturer

Samples which showed positive trends in AUC with concentration of the particles included sucrose, glucose, native starch, and wax. No trend was observed in samples made with: pre-gelatinized starch, MCC pellets, glass beads, and diamond. These results indicate that for an interaction to exist between the particle and EC the particle must contain a glucose moiety with its O(2)-H group (Figure 4.2C) available for interaction. Sucrose, glucose, native starch, and pre-gelatinized starch are all known to have the O(2)-H group participating in intermolecular hydrogen bonds. There must be enough free OH groups or the existing intermolecular hydrogen bonds must be weak enough to allow for interaction of the particles with EC. Furthermore, since EC does not seem to interact with pre-gelatinized starch, it is likely necessary that the particle have an organized, crystalline structure to be able to interact with EC. Pre-gelatinized starch lacks crystallinity since it has been lost during the gelatinization process.
In the case of cellulose pellets, glucose moieties are present; however, the O(2)-H group is not abundantly available for interaction with EC. Cellulose is very tightly packed with many strong hydrogen bonds holding the molecules together including intra- and intermolecular hydrogen bonds involving the O(2)-H group.\textsuperscript{54} For these reasons cellulose is not able to interact with EC.

The sample made with RBW showed a trend of increasing hardness with increasing concentration of particles. However, RBW does not contain a glucose moiety and would not be expected to interact with EC in the same way that EC interacts with sucrose. It was shown in the section on MD simulations that EC is able to hydrogen bond at the ester groups with both POPC and triglycerides. A similar interaction may be present in the sample made with RBW since RBW also contains an ester linkage. Further mechanisms may also be at play in the RBW sample. RBW is somewhat soluble in EtOH\textsuperscript{55} and RBW can gel vegetable oil.\textsuperscript{56} All of these factors may contribute to structure formation within the sample that could increase the value for the AUC as the concentration of RBW increased.

There was no trend observed for samples made with glass beads or diamonds. This was expected since glass beads are non-crystalline and diamonds do not contain hydroxyl groups which could interact with EC.
Figure 4.10 Area under the displacement in compression curve at 40°C for samples made with various particles at increasing particle concentration and constant EC concentration. (A) sucrose; (B) glucose; (C) native starch; (D) pre-gelatinized starch; (E) MCC pellets; (F) MCC fibers; (G) RBW; (H) glass beads; (I) diamond. Values represent the average and standard deviation. Black squares are the particles treated with EC in EtOH and grey circles are the control particles treated with only EtOH.

4.3.3 Fourier transform infrared spectroscopy

The FTIR spectra collected had a resolution of 2 cm\(^{-1}\) and a data spacing of 1 cm\(^{-1}\), therefore, a peak shift of 1 cm\(^{-1}\) or more is considered significant. There were very few significant peak shifts.
observed. However, one shift that was consistent and significant occurred at around 3335 cm\(^{-1}\). This peak is unique to sucrose in the multi-component systems tested. Some peak shifts were also observed in the fingerprint region of the spectra for the heat samples analyzed by ATR. The average change in peak position (ΔPP) and standard deviation for significant peak shifts are shown in Table 4.4.

All samples without lecithin showed the significant peak shift at 3335 cm\(^{-1}\), although the shift was generally only one wavenumber. This peak is associated with stretching of the hydroxyl group on carbon two of the glucose ring on the sucrose molecule\(^{49}\) (Figure 4.2A). This O(2)-H hydrogen in sucrose crystals participates in intermolecular hydrogen bonds with the oxygen on carbon 6 of the fructose moiety (O’(6)). Since the shift at 3335 cm\(^{-1}\) is to higher wavenumbers it is thought that this is indicative of weakening or loss of this intermolecular hydrogen bond when EC is present. We propose that the O(2)-H would then be available for hydrogen bonding with EC. This seems probable since the molecular simulations have shown that EC can hydrogen bond with sucrose and the hardness tests have also indicated an interaction between EC and sucrose.

The heat method samples with 2% or 5% EC also showed peak shifts to higher wavenumbers at 1346 cm\(^{-1}\) and to lower wavenumbers at 1322 and 1105 cm\(^{-1}\). Shifts at 1346 and 1322 cm\(^{-1}\) are associated with bending of COC and COH moieties on the sucrose respectively. The shift at 1105 cm\(^{-1}\) is attributed to stretching of a CO group. This is further indication of interactions of sucrose with EC that have lead to small changes in the molecular structure of the sucrose. These shifts were not observed in the solvent substitution samples. This may be because canola oil has peaks at similar wavenumbers and these peaks may be confounding our results. However, differences in sample preparation and analytical techniques may also be an explanation for the lack of shifts observed in this region for the solvent substitution samples.

Interestingly, there were no peak shifts observed in heat method samples made with lecithin coated sucrose. The presence of lecithin at the surface of sucrose prevented any bonding between sucrose and EC likely due to a shielding effect which kept the EC at too great a distance from the sucrose for interactions to occur. Again, this result confirms what was seen in the simulations and the hardness tests.
Table 4.4 Peak position (PP) and average peak shifts (ΔPP) with respect to the EC-free control observed in solvent substitution and heat method samples of sucrose with EC. The error is the standard deviation of three replicates.

<table>
<thead>
<tr>
<th>Samples</th>
<th>PP (cm⁻¹)</th>
<th>ΔPP (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent substitution method</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16% EC on sucrose (EtOH) rep 1</td>
<td>3335</td>
<td>1 ± 0.05</td>
</tr>
<tr>
<td>16% EC on sucrose (EtOH) rep 2</td>
<td>3335</td>
<td>1 ± 0.09</td>
</tr>
<tr>
<td>33% EC on sucrose (EtOH)</td>
<td>3335</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>50% EC on sucrose (EtOH)</td>
<td>3335</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td><strong>Heat method</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% EC on sucrose in canola oil</td>
<td>3335</td>
<td>2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1346</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1322</td>
<td>-1 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>1105</td>
<td>-1 ± 0.5</td>
</tr>
<tr>
<td>5% EC on sucrose in canola oil</td>
<td>3335</td>
<td>1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1346</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>1322</td>
<td>-1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1105</td>
<td>-2 ± 0.1</td>
</tr>
</tbody>
</table>

*Negative numbers represent shifts to lower wavenumbers*

**4.3.4 Scanning electron microscopy**

Images of the defatted chocolates (Xerogels) are shown in Figure 4.11. From the photographs at the top of the figure it is clear that the EC- and EtOH-containing samples held their shape throughout the defatting procedure and were able to be picked up with the tweezers with little structural damage. However, the control sample almost completely disintegrated since there was no secondary structure to hold its shape when the primary structure of solid fat was removed. Much of the solids fell through the mesh and to the bottom of the beaker. A small chunk of the control sample was left on the mesh and was able to be imaged.

It can be seen from the first row of SEM images that there are some slight differences observed between the samples. The control sample had a very smooth region with a rougher ledge to the upper right. The smooth region likely corresponds to an area where the structure has collapsed upon itself. This region would best represent the majority of the control chocolate structure considering most of the chocolate disintegrated during sample preparation. In contrast, both the EtOH control and EC sample show well defined structures with the EC sample being somewhat...
rouder and better defined. Perhaps this indicates that the solid particles are more tightly held together in the EC sample. Both structures have pores and crevices where the fat would be located. The next row at a higher magnification shows slightly more detail of the above mentioned features. All samples show the smooth, straight edges of the large sucrose crystals which can be distinguished from the smaller, rough particles of cocoa powder and milk proteins. The bottom row shows a very high magnification image of the samples. Again, large sucrose particles and smaller cocoa powder and milk protein particles are visible in the control sample with a loosely piled arrangement and no apparent inter-particle structure. The EtOH control shows large sucrose crystals with other smaller particles closely packed together. There were small cocoa powder and milk protein particles and neighbouring sucrose particles stuck to all surfaces of the sucrose particles. The EC sample looked very similar to the EtOH control but displaying perhaps more aggregation. Clearly both EtOH and a combination of EC in EtOH were able to create a secondary network of solid particles within the chocolate. When the fat was removed this structure was left largely intact and would be responsible for the heat resistance in these chocolates.
Figure 4.11 Photographs and SEM images of defatted chocolate samples at various magnifications. Columns show: (A) control; (B) EtOH control; and (C) 2.17% EC in chocolate. Sucrose is denoted as “s” and milk protein or cocoa powder is denoted by “p”
4.4 Conclusions

In this study, we show by multiple methods, that EC and sucrose can interact. MD simulations and FTIR have indicated that these interactions are likely due to hydrogen bonding between polar groups on the EC and sucrose. These interactions lead to structure formation within chocolate samples and this structure was responsible for heat resistance. It was also found that when lecithin was added to the system as a coating on the sucrose crystals hydrogen bonding between EC and sucrose was hindered. This resulted in a sample with reduced heat resistance due to the lack of a sucrose-EC network. Finally, the interactions between EC and sucrose were not unique; network formation also occurred when glucose or native starch was used. A requirement was that the particle must be crystalline in nature and contain a glucose moiety with free O(2)-H groups for interactions with EC to take place.

References


CHAPTER 5

The role of lecithin and solvent addition in ethylcellulose-stabilized heat resistant chocolate

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Abstract

Large deformation mechanical testing and Fourier-transform infrared spectroscopy were used to gain further insights into the mechanism of heat resistance in ethylcellulose- (EC) stabilized chocolate prepared using the solvent substitution method (Chapter 4). Here we show that the presence of lecithin at the surface of sucrose reduced heat resistance by impeding interactions between EC and sucrose. These techniques along with fluorescence microscopy also showed that the EtOH used in solvent substitution chocolate was able to remove the EtOH soluble lecithin phospholipids from the surface of the sucrose. Removal of the lecithin and the slight solubilization of sucrose in EtOH both have positive impacts on heat resistance. It was also found that EtOH may reduce heat resistance by destabilizing the casein micelle in samples made with skim milk powder. Finally, results have indicated that EC is likely able to interact with the lactose in skim milk powder and the starch in cocoa powder leading to greater heat resistance. These findings will be useful in developing the ideal heat resistant chocolate formula.

5.1 Introduction

Previous work has shown that the ethylcellulose (EC) solvent substitution method can produce heat resistance in chocolate \cite{1} by the creation of an EC-sucrose network within the chocolate (Chapter 4). EC solvent substitution heat resistant chocolate was produced by mixing 10\% of a 20\% EC ethanolic solution with molten chocolate and evaporating the ethanol (EtOH) after the chocolate had hardened \cite{1}. The resulting chocolate contained 2.17\% EC and had a hardness of 18 N at 40°C when tested with a large deformation mechanical tester using a two-plate compression geometry. It was determined by a variety of methods that EC is able to interact with the sucrose in chocolate, forming a network that resists deformation at high temperatures, 40°C in our case, where that fat has liquefied. This work also showed that EtOH has a small but
significant enhancing effect on the heat resistance of this chocolate. The current research will focus on better understanding this effect of EtOH on the heat resistance of chocolate.

Previous research has lead to the development of heat resistant chocolate by the addition of small amounts of water [2]. The water dissolves a portion of the sucrose surface causing the crystals to become sticky and aggregate with the solid particles in the chocolate such as other sucrose crystals, or cocoa or milk powder. The aggregated solids form a network that is able to trap liquefied fat at elevated temperatures [3]. A similar mechanism may be responsible for the heat resistance produced by addition of EtOH. At 37°C the solubility of sucrose in water is 0.701 g/g solution while the solubility of sucrose in EtOH is 1000 times lower at 0.0007 g/g solution [4]. This small degree of solubility of sucrose in EtOH may allow for some network formation in the chocolate. Beneficially, this low solubility lessens particle aggregation in the molten chocolate during manufacture which is a major problem when trying to add water to chocolate, a phenomenon called seizure [5]. Ethyl acetate will be used for comparative purposes since it can dissolve the EC but not the sucrose. The solubility of sucrose in ethyl acetate is around 3.21 mg/L solution [6] at 50°C which is approximately 3.6×10^{-6} g/g solution. It was previously shown that the heat resistance of chocolates made with ethyl acetate was much lower than those made with EtOH [1]. Experiments will be carried out to explore if sucrose solubility in EtOH can be used to explain these heat resistance results.

The effect of solvents on the other major ingredients in chocolate will also be examined. It is known that EtOH can destabilize casein micelles [7] however, it is not clear if this impacts the heat resistance of chocolate containing milk proteins. Furthermore, the effect of EtOH on cocoa solids is unknown and will therefore be explored.

Chocolate is typically manufactured with 0.3-0.5% lecithin used as an emulsifier to coat the sucrose crystals with phospholipids which allows for better flow properties in the chocolate [8]. It is therefore of interest to understand the effects of the sucrose crystals being coated in lecithin and used to produce EC solvent substitution heat resistant chocolate. Lecithin contains a large amount of phospholipids, one of the major ones being phosphatidylcholine [8]. Atomic scale molecular dynamics simulations and Fourier-transform infrared (FTIR) spectroscopy showed that a phosphatidylcholine coating on sucrose crystals prevents EC from interacting with the
sucrose by keeping the EC at too far a distance from the sucrose for hydrogen bonding to occur (Chapter 4). It was also shown that this lack of interaction between EC and sucrose hinders heat resistance. However, these experiments did not account for the EtOH present in solvent substitution chocolate. Considering phosphatidylcholine is soluble in EtOH [9] it was hypothesized that the EtOH present in solvent substitution chocolate is able to remove some of the lecithin phospholipids from the surface of the sucrose. This hypothesis along with the consequences it has on heat resistance of chocolate will be investigated.

5.2 Materials and methods

5.2.1 Hardness tests of model systems

Model chocolate samples were prepared using various chocolate ingredients. Palm kernel oil (PKO) (Nealander’s International Inc., Mississauga, ON) was used as the fat phase since it is similar to cocoa butter but does not require tempering to achieve the correct crystal polymorph. Granulated sucrose (Red Path, Toronto ON) was chopped into a powder using a blender. Other ingredients included: EC 10 cP (Dow Chemicals, Midland, MI), soybean lecithin (Grain Process Enterprises Ltd. Scarborough, ON), 20% fat cocoa powder (Metro Inc., Montréal, QC), skim milk powder (Nealander’s International Inc., Mississauga, ON), absolute EtOH (Commercial Alcohols, Brampton, ON) and ethyl acetate (Sigma Aldrich, St. Louis, MO).

Samples were first prepared with only sucrose, PKO, EC and optionally lecithin. The concentration of sucrose was increased from 21.7-76.1% while the final concentration of EC was held constant at 2.17%. Controls were prepared with no sucrose; these samples contained the same ratio of EC:PKO as the equivalent sample with sucrose. Lecithin was added at levels corresponding to what would typically be found in a chocolate. The ratio of lecithin to solids was held constant and calculated assuming lecithin and solids-non-fat concentrations in chocolate of 0.3% and 70% respectively [8, 10]. A sample preparation method similar to the solvent substitution method was used where EC was added to the system as a 20% solution in either EtOH or ethyl acetate. The EC was mixed with PKO crystals for the sucrose-free control for 1 min at 200 rpm using a stirrer with a paddle impeller. Samples with sucrose were mixed with the PKO prior to addition of the EC. Lecithin was added to the molten PKO if used. Immediately following mixing the samples were moulded and then cooled at 5°C in a refrigerator for 20-30
min. Samples in the form of tablets (3.60 x 1.90 x 0.68 cm) were removed from the moulds after cooling. Samples were left at room temperature overnight and then placed in an incubator at 30°C for approximately 9 days to evaporate the solvent.

Further experiments were carried out using the PKO and sucrose as well as the other major chocolate ingredients, skim milk powder and cocoa powder. Crude milk, white, and dark chocolate samples were prepared along with samples containing only skim milk powder or cocoa powder as the solids-non-fat. The formulas used are shown in Table 5.1. The samples were prepared by mixing the ingredients in a countertop stand mixer at medium power with an attached circulating water bath set at 50°C until well mixed. The 20% EC solution or just the solvent was then added to the samples and mixed briefly. Samples were moulded, cooled, and demoulded and the solvent evaporated as above. The samples were all made with 8% solvent; those with EC would contain 2.17% EC following evaporation of the solvent.

Finally, a commercially available compound milk chocolate (Bulk Barn, Richmond Hill, ON) was melted and mixed by hand with increasing proportions of EtOH. The chocolate was moulded, cooled, demoulded as above and the EtOH was thereafter evaporated in an incubator at 30°C for 9 days.

All samples were tested for heat resistance using a texture analyser (Stable Micro Systems Ltd., Surrey, UK). A cylindrical probe with a 1.80 cm diameter was used with a set displacement in compression protocol. The samples were incubated at 40°C for 2 h and then compressed 4 mm. The force at 2 mm displacement was taken as a measure of the heat resistance of the sample.

5.2.2 Scanning electron microscopy (SEM)

Defatted chocolate samples were prepared to be imaged using an SEM. Compound milk chocolate (Bulk Barn, Richmond Hill, ON) was used to produce EtOH control samples with 2, 4, 6 or 8% EtOH where 8% EtOH was equivalent to the amount of EtOH in typical EC solvent substitution chocolate samples. The EtOH was added to the molten chocolate and mixed briefly by hand. Samples were moulded, cooled at 5°C for 20-30 min, then demoulded. The EtOH was thereafter evaporated during storage in a 30°C incubator for 9 days. The fat was removed from the samples by immersion in a hexane bath for two days. The fat-free samples were removed from the hexane and stored at room temperature for any residual solvent to evaporate. The
samples were then cut and mounted onto a sample holder with the cut face up. An Emitech K550 Sputter Coater (Quorum Technologies Ltd., East Grinstead, UK) was used to apply a 20 nm gold coating [11] to the samples. An S-570 SEM (Hitachi High-Tecnologies, Tokyo, Japan) with Quartz PCI image capture software (Quartz Imaging Corporation, Vancouver, BC) were used to obtain images of the samples.

Table 5.1 Formulas of samples used in the hardness tests

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fat</th>
<th>Sugar</th>
<th>Cocoa solids</th>
<th>Skim milk powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk powder</td>
<td>45*</td>
<td>0</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Cocoa powder</td>
<td>40*</td>
<td>0</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>White</td>
<td>32</td>
<td>48</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Milk</td>
<td>32</td>
<td>48</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Dark</td>
<td>32</td>
<td>48</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

* These samples required more fat to achieve a well mixed, smooth sample

5.2.3 Karl Fischer titration

The materials used in the production of heat resistant chocolate were tested for water content to ensure that no water was being added to the chocolate and skewing results. Samples of ground sucrose, EtOH, ethyl acetate, and 20% EC 10 cP dissolved in EtOH or ethyl acetate were added at levels of 10% to methanol. The samples were vortexed for 1 min. Undiluted samples of EtOH and ethyl acetate were also tested. Care was taken to avoid any absorption of water from the air. The samples were analyzed by Karl Fischer titration using a 701 KF Titrino automatic titrator (Metrohm Ltd., Herisau Switzerland) and the standard operating procedure for the titrator [12]. The Karl Fischer titration measures trace amounts of water through a reaction based on the oxidation of sulfur dioxide by iodine in the presence of water.

5.2.4 Fourier transform infrared (FTIR) spectroscopy

Samples were prepared for analysis by FTIR spectroscopy using methods similar to the solvent substitution method described above. Sucrose was ground to a fine powder using a blender and a mortar and pestle. Lecithin coated sucrose was prepared by first dissolving 1% soybean phospholipids (Sigma-Alrich, Saint Louis, MO) with a content of 14-23% phosphatidylcholine in chloroform. The dissolved phosphatidylcholine was mixed with sucrose using an overhead mixer
at levels that would typically be found in a chocolate which was calculated to be 0.6% by weight phospholipids on the sucrose. The chloroform was then evaporated.

Samples were prepared by mixing the 20% EC in EtOH or ethyl acetate solution with sucrose or lecithin coated sucrose by hand. EC was added to achieve samples with 16, 33, or 50% EC after removal of the solvent. The sample was continually mixed as the solvent was evaporated and the dried sample was then incubated in an oven at 80°C for 1 h. A mortar and pestle was then used to grind the sample to a fine particle size. The incubation period of 80°C for 1 h was then repeated to ensure complete removal of the solvent. Samples were cooled and stored in a desiccator. Control samples were also made using the solvents but without the EC. These powder-type samples were analysed using a Thermo Fisher Scientific Nicolet 6700 FTIR spectrometer (Waltham, MA) with deuterated triglycine sulfate (DTGS) detector in transmission mode. The spectra were collected as KBr pellets at 64 scans and had a resolution of 2 cm\(^{-1}\). Each sample was analyzed in triplicate.

OMNIC Spectra Software (Thermo Electron Scientific Instruments Corporation, Madison WI) was used for analysis of the spectra. The spectra were first ratioed to the background spectra of a sample-free KBr pellet. The peak positions were then recorded for all samples and controls. Peaks for sucrose that have been identified in the literature [13] were of particular interest. The average peak position for the EC-free control was subtracted from the peak position for the sample and this value was recorded as the peak shift. The experimental setup allowed for peak shifts as small as 1 cm\(^{-1}\) to be considered significant. These significant peaks were then identified.

5.2.5 Fluorescence microscopy

Phosphatidylcholine labeled along the acyl chain with nitrobenzoxadiazole (1-oleoyl-2-[12-((7-nitro-2-1,3-benzoxadiazol-4-yl)amino)dodecanoyl]-sn-glycero-3-phosphocholine) was obtained from Avanti Polar Lipids Inc. (Alabaster, AL). The excitation and emission maxima for this fluorophore were 460 and 534 nm respectively. This fluorescent phosphatidylcholine has similar solubility to phosphatidylcholine making it ideal for approximation of the behaviour of phosphatidylcholine. The phosphatidylcholine was first dissolved in chloroform to a concentration of 0.4 mg/mL. A mix of 0.1 g of the phosphatidylcholine solution, 6.67 g of canola
oil, and 3.33 g of granulated sucrose crystals was added to a test tube. The contents were mixed by vortex for 10 min. This control sample was split into 1 g portions in four more test tubes. A 1 g aliquot of EtOH, ethyl acetate, or 10% EC 4 cP in EtOH or ethyl acetate was added to the test tube and vortexed for 1 min. The samples were then spread onto glass slides and left briefly for the solvent to evaporate. Care was taken to avoid exposing the fluorescent phosphatidylcholine and samples to heat or light to avoid photobleaching. A drop of canola oil and cover slips were placed on the samples just prior to viewing. An Olympus BX60 light microscope (Richmond, ON) equipped with an MWIB fluorescence filter cube (excitation filter at 460-490 nm and emission filter > 515 nm) and DP71 camera along with Image Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD) were used to obtain micrographs of the samples. Micrographs were first taken in bright field and then the system was switched to fluorescence mode under the same field of view. Micrographs of sucrose taken in fluorescence mode were all taken with an exposure time of 11.405 s. A control system replacing sucrose with silica glass beads (Potters Industries LLC, Valley Forge, PA) was also tested with the EtOH and ethyl acetate treatments. An exposure time of 18.761 s was necessary for these samples. Micrographs were transformed into greyscale using Adobe Photoshop CS5 software (Adobe Systems, San Jose, CA).

The intensity of the fluorescence of the nitrobenzoxadiazole-labelled phosphatidylcholine in the various solvents used was measured using a RF-5301PC spectrofluorophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) to ensure the solvents did not impact the fluorophore. The phosphatidylcholine was first diluted in chloroform to 0.2 mg/mL. This sample was then aliquoted into foil-wrapped test tubes and the chloroform was evaporated. The phosphatidylcholine was then dissolved in chloroform, EtOH, or ethyl acetate in increasing dilution. The test tubes were sealed to ensure no evaporation of the solvents and then vortexed. Samples were placed in a quartz cuvette with lid prior to testing. The fluorometer was set to excite the sample at 460 nm and to measure the fluorescence at 534 nm. The test was executed in low sensitivity mode with a slit width of 1 which allowed for most of the dilutions to be tested without reaching the intensity limit of the fluorometer.

5.2.6 Residual EtOH

The amount of residual EtOH in solvent substitution heat resistant chocolate was measured using an enzyme assay. Samples were prepared in triplicate using the solvent substitution method with
compound milk chocolate (Bulk Barn, Richmond Hill, ON). Samples included a control, a control with 8% EtOH, and 2.17% EC cP 45 (Dow Chemicals) from a 20% solution in EtOH. The EC sample contained 8% EtOH prior to evaporation. Three chocolates were placed in tin containers and incubated at 30°C for 14 days. Following the incubation period the samples were prepared for the enzyme kit using procedures suggested in the kit manual for solid, fat-containing samples [14]. Chocolates were placed between plastic wrap and crushed with a hammer and then chopped with a knife into very fine pieces while in a fridge at 5°C to prevent any EtOH evaporation. A 5 g aliquot of sample was weighed into a 100 mL volumetric flask. Approximately 90 mL of 70°C water was added to the flask and the flask was sealed with parafilm and mixed to extract the EtOH present. An internal standard was prepared using some of the control chocolate with 0.01 g of EtOH added to the flask. Samples were left to cool at room temperature overnight. Once cool the flasks were topped up to 100 mL and mixed again. The samples were filtered using a Whatman No. 1 filter paper and then stored in capped vials. The Ethanol enzyme kit from Megazyme International Ireland (Bray, Ireland) with the manual assay procedure was performed on the samples according to the manufacturer’s directions [14]. This kit utilizes the two reactions shown in Equations 5.1 and 5.2:

\[
\text{EtOH} + \text{NAD}^+ \xrightarrow{(\text{ADH})} \text{acetaldehyde} + \text{NADH} + H^+ \tag{5.1}
\]

\[
\text{acetaldehyde} + \text{NAD}^+ + H_2O \xrightarrow{(\text{Al-DH})} \text{acetic acid} + \text{NADH} + H^+ \tag{5.2}
\]

In the first reaction EtOH is oxidized by nicotinamide-adenine dinucleotide (NAD\(^+\)) to acetaldehyde and this is catalyzed by the enzyme alcohol dehydrogenase (ADH). The second reaction utilizes the enzyme aldehyde dehydrogenase (Al-DH) and NAD\(^+\) to oxidize acetaldehyde to acetic acid. Both reactions produce one NADH molecule therefore the amount of NADH formed is twice the amount of EtOH consumed. NADH was measured by the increase in absorbance of the sample at 340 nm. A Beckman DU 7400 spectrophotometer (Beckman Coulter Inc., Brea, California) was used to measure the absorbance of the samples. The concentration of EtOH in the samples was calculated using the Beer-Lambert law:

\[
c = \frac{V \times MW}{e \times d \times v \times 2} \times \Delta A \tag{5.3}
\]
where \( V \) is the final volume, MW is the molecular weight of ethanol, \( \varepsilon \) is the extinction coefficient of NADH at 340 nm (6300 L/mol×cm), \( d \) is the light path, \( v \) is the sample volume, and \( \Delta A \) is the difference in absorbance from the start of the reaction to the end of the reaction for the sample minus that for the blank.

### 5.3 Results and discussion

#### 5.3.1 Hardness tests of model systems

Results of the hardness tests for samples made with either EtOH or ethyl acetate are shown in Figure 5.1 and Figure 5.2 respectively. It is clear that the addition of lecithin greatly decreased the hardness of the samples and controls for the both solvent methods. The same was true for samples made using a solvent free method (Chapter 4). The sample prepared with an excess of lecithin on sucrose with EC (Figure 5.1) showed no exponential increase in hardness with increasing sucrose concentration. Instead the hardness stayed relatively the same across an extensive range of sucrose concentrations. This indicated that an excess of lecithin in the solvent substitution samples with EtOH led to a complete loss of interactions between EC and sucrose. This was likely due to the shielding effect caused by the boundary layer of lecithin on the sucrose surface. The EC was not able to get close enough to the sucrose to interact. This was previously predicted by atomic scale molecular dynamics simulations (Chapter 4).

We hypothesized that EtOH is able to remove lecithin from the surface of the sucrose crystals, allowing for interactions between EC and sucrose to take place. In Figure 5.1 it was observed that the sample with lecithin was still softer than the sample without lecithin despite EtOH being present. To determine if the EtOH had a positive effect on hardness of samples with lecithin, the solvent substitution samples made with EtOH were compared to those made with ethyl acetate since ethyl acetate should have no effect on the lecithin. From the data it was estimated that samples with sucrose and EC from EtOH were generally 1.7 times harder than those with EC from ethyl acetate. This was attributed to the slight solubility of sucrose in EtOH. Interestingly, samples with sucrose, lecithin, and EC from EtOH were generally 3.7 times harder than those with EC from ethyl acetate. We believe that this extra difference in hardness is good evidence that EtOH is removing some lecithin from the sugar surface and this leads to a harder sample. It is likely that not all of the lecithin phospholipids were removed with the EtOH since only some
of the phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, are soluble in EtOH [15]. This would explain why the hardness of samples made with lecithin and EtOH remained lower than those samples without lecithin despite some of the phospholipids having been removed.

![Figure 5.1](image1.png)

**Figure 5.1** Hardness of model chocolate samples made using the solvent substitution method with sucrose (suc), EC, lecithin (lec) and EtOH as the solvent

![Figure 5.2](image2.png)

**Figure 5.2** Hardness of model chocolate samples made using the solvent substitution method with sucrose (suc), EC, lecithin (lec) and ethyl acetate as the solvent

Further experiments revealed the effect of solvents on skim milk powder and cocoa powder, the other major ingredients in chocolate. Results in Figure 5.3 show that both EtOH and ethyl acetate have a negative effect on the skim milk powder and white chocolate samples. This suggests that perhaps these solvents have caused some destabilization of the casein micelle [7] and that this
may have a negative impact on heat resistance. The milk chocolate sample which contained some skim milk powder did not appear to be affected by the solvents. Perhaps this is due to the presence of cocoa powder in this sample. The dark chocolate sample, which contained no skim milk powder, was also unaffected by the solvents. The EtOH may have had a positive effect on the cocoa powder. However, this was not apparent in the milk or dark chocolate. There was also a negative effect of solvent addition on the skim milk powder samples that contained EC. All other samples were harder than their respective controls when EC was added. Interestingly, the cocoa powder sample which contains no sucrose showed heat resistance when EC was added. This is likely due to the large amount of starch naturally present in cocoa powder [16], which might be interacting with the EC. It has been shown that EC can interact with native starch likely in a similar way that EC interacts with sucrose since the structures of sucrose and starch are similar (Chapter 4). Similarly, there could be interactions between EC and the lactose present in skim milk powder since lactose also has a similar structure to sucrose. It was also found that samples that contained sucrose and were made with EC in EtOH showed a greater hardness than those made with EC in ethyl acetate. This is likely because EtOH can dissolve some of the sucrose in these samples while the ethyl acetate cannot.

It should be noted that in solvent substitution chocolate the solvent contains 20% EC and therefore, it is unlikely that all of solvent in this mix is available to interact with other species in the chocolate. Therefore, all solvent-treated controls were likely made with an excess amount of free solvent. This is important since the effect of the solvent on hardness of the samples was found to be concentration dependent (Figure 5.4). A trend of increasing hardness with concentration of EtOH up to 7% was observed in compound milk chocolate. This trend was likely due to the greater amounts of EtOH being able to dissolve a greater amount of the sucrose which was able to create a stronger solid particle network in the chocolate. It is thought that the decrease in hardness above 7% EtOH might be indicative of a weaker network being formed when there was too great of a proportion of solvent in the chocolate. The crevices and pores created by the evaporating solvent could potentially weaken the solid particle network in the chocolate. The decrease in hardness could also be indicative of too much dissolution of the sucrose.
Figure 5.3 Hardness at 40°C of chocolates and ingredients treated with EtOH or ethyl acetate
Figure 5.4 Hardness at 40°C of compound milk chocolate treated with EtOH

5.3.2 Karl Fischer titration

All of the samples tested showed no trace of water. The lower detection limit of the equipment was 500 μg of water [12] which would correspond to around 1% water by weight in the solvents tested. Therefore, for samples made with 8% solvent a maximum of 0.08% water would be added to the chocolate. It is probable that this amount of water would be far too small to have any significant effect on heat resistance.

5.3.3 Scanning electron microscopy

Figure 5.5 shows the structure left behind in defatted, compound milk chocolates treated with increasing amounts of EtOH. All of the samples look fairly similar. There was a clear structure that appeared somewhat smooth at low magnifications. The higher magnifications show the sharp edges of the large sucrose crystals with smaller, rougher particles of cocoa powder and milk proteins attached. Although not obvious in the images, while handling the samples it was clear that as the concentration of EtOH increased, the structure became stronger, less fragile, and easier to handle. The sample with 2% EtOH was very crumbly and it was difficult to obtain an intact piece to image. The 8% sample was almost completely intact following the defatting procedure and could be handled with little destruction to the structure. This mimics the result observed in Figure 5.4 and was likely caused by a greater proportion of the sucrose being dissolved in the higher concentrations of EtOH as discussed previously.
4.3.4 Fourier transform infrared spectroscopy

It was shown previously (Chapter 4) that addition of EC to sucrose led to a peak shift of about 1 cm\(^{-1}\) at 3335 cm\(^{-1}\) corresponding to the stretching of the hydroxyl group on carbon two (O(2)-H) of the glucose ring of the sucrose molecule [13]. It was concluded that this peak shift was indicative of a loss of intermolecular hydrogen bonding between O(2)-H on the glucose moiety of one sucrose molecule with the O’(6) on the fructose moiety of another sucrose molecule. Thus it is probably that the O(2)-H group would then be available to hydrogen bond with EC. The results in Table 5.2 show that the same shift was also observed in sucrose samples that were made with ethyl acetate instead of EtOH. This was expected since EC dissolved in ethyl acetate
has been shown to be effective in producing heat resistance through sucrose-EC network formation in chocolate [1] and model chocolate systems.

Interestingly, when sucrose coated with phosphatidylcholine was used, a peak shift was observed for samples made with EC in EtOH but not for EC in ethyl acetate. A shift was not observed in the ethyl acetate samples likely because phosphatidylcholine has poor solubility in ethyl acetate [17]. The phosphatidylcholine remaining at the surface of the sucrose in the ethyl acetate samples created a barrier that kept the EC at a distance too far from the sucrose for interactions to occur. This was also seen by FTIR in similar phosphatidylcholine-coated sucrose samples made using a solvent-free method and was predicted by atomic scale molecular dynamics simulations (Chapter 4). In contrast the good solubility of phosphatidylcholine in EtOH [9] allowed the phosphatidylcholine to be removed from the surface of the sucrose freeing up the surface for interaction with EC. These results are clear indications that the presence of lecithin at the surface of sucrose disrupts bonding between sucrose and EC. Furthermore, the EtOH in solvent substitution samples is able to remove phosphatidylcholine from the surface of the sucrose allowing for interactions between sucrose and EC. These results correspond well with the results for hardness tests of model systems described above and show that the disruption of sucrose-EC bonding reduces heat resistance of the samples.

Table 5.2 Peak position (PP) and average peak shifts (ΔPP) with respect to the EC-free control observed in solvent substitution samples containing phosphatidylcholine (PC) coated sucrose made with EtOH or ethyl acetate. The error is the standard deviation of three replicates

<table>
<thead>
<tr>
<th>Samples</th>
<th>PP (cm⁻¹)</th>
<th>Δ PP (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EtOH samples</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16% EC on sucrose (Chapter 4)</td>
<td>3335</td>
<td>1 ± 0.05</td>
</tr>
<tr>
<td>16% EC on PC coated sucrose</td>
<td>3335</td>
<td>2 ± 0.07</td>
</tr>
<tr>
<td>33% EC on PC coated sucrose</td>
<td>3335</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>50% EC on PC coated sucrose</td>
<td>3335</td>
<td>1 ± 0.06</td>
</tr>
<tr>
<td><strong>Ethyl acetate samples</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16% EC on sucrose rep 1</td>
<td>3335</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td>16% EC on sucrose rep 2</td>
<td>3335</td>
<td>1 ± 0.03</td>
</tr>
<tr>
<td>16% EC on PC coated sucrose rep 1</td>
<td>3335</td>
<td>1 ± 0.03</td>
</tr>
<tr>
<td>16% EC on PC coated sucrose rep 2</td>
<td>3335</td>
<td>1 ± 0.03</td>
</tr>
</tbody>
</table>
5.3.5 Fluorescence microscopy

Micrographs of fluorescent phosphatidylcholine-coated sucrose crystals treated with solvents are shown in Figure 5.6. The sucrose control sample clearly shows the fluorescing phosphatidylcholine as a bright coating on the surface of the sucrose. Under bright field the sucrose sample treated with EtOH showed some clumping of the crystals and less sharp edges which may indicate some dissolution of the sucrose in the EtOH. There also appeared to be bridges between some of the sucrose crystals (indicated by the arrows). The image of this sample under fluorescence clearly showed a decrease in brightness compared to the control and this was thought to be associated with a loss of phosphatidylcholine from the surface of the sucrose crystals. In contrast, the sample treated with ethyl acetate looked very similar to the control; it did not show clumping of or connections between the sucrose crystals and also had very similar brightness to the sucrose control. This indicates that the ethyl acetate did not dissolve any appreciable amount of the sucrose and was not able to remove phosphatidylcholine from the surface of the sucrose due to the insolubility of phosphatidylcholine in this solvent.
Figure 5.6 Micrographs of sucrose crystals coated in fluorescent phosphatidylcholine in oil. Micrographs A, C, and E were taken under bright field and B, D, and F were taken using fluorescence mode. Figures A and B show the control sample with no solvent; C and D show the sample treated with EtOH; and E and F show the sample treated with ethyl acetate. Arrows indicate areas where sucrose crystals appear to have fused.
Similar results were observed when EC was dissolved in the solvent and added to the sucrose (Figure 5.7). The sample made with EC in EtOH showed some dissolution and clumping of the sucrose crystals. The fluorescent micrograph showed that much of the phosphatidylcholine moved from the sucrose surface and into the surrounding areas filled with oil and EC. The sucrose appeared as dark phosphatidylcholine-depleted crystals in a sea of phosphatidylcholine. The sample made with EC in ethyl acetate showed perhaps some clumping, however, in fluorescence mode it was obvious that all of the phosphatidylcholine remained at the surface of the sucrose. Again, this indicates that EtOH was able to dissolve a small portion of the sucrose and remove phosphatidylcholine from the surface of the sucrose but ethyl acetate was not able to do so.

Figure 5.7 Micrographs of sucrose crystals coated in fluorescent phosphatidylcholine in oil. Micrographs A, and C were taken under bright field and B, and D were taken using fluorescence mode. Figures A and B show the sample treated with EC in EtOH; and C and D show the sample treated with EC in ethyl acetate
The control samples prepared with glass instead of sucrose showed the same results as the sucrose samples (Figure 5.8). The exposure was increased for these samples compared to the sucrose samples. This was necessary to easily view the beads and may be due to a lesser driving force for the phosphatidylcholine to go to the surface of the inert glass beads compared to the polar sucrose surface. The control with no solvent showed phosphatidylcholine at the surface of the glass beads. The addition of EtOH resulted in a less bright micrograph compared to the control which likely indicates a loss of phosphatidylcholine from the surface of the beads. Again, the addition of ethyl acetate did not reduce the brightness of the sample compared to the control and therefore ethyl acetate was not able to wash away the phosphatidylcholine. These controls also indicate that removal of phosphatidylcholine from the surface of a particle by EtOH is due to solubility of the phosphatidylcholine in EtOH and not solubility of the particle in EtOH.

Furthermore, the results for fluorescence intensity of the nitrobenzoxadiazole-labelled phosphatidylcholine in various solvents confirm that the loss of brightness observed was due to removal of phosphatidylcholine and not a result of the solvent interfering with the fluorescent activity of the phosphatidylcholine (Figure 5.9). The phosphatidylcholine showed a linear increase in intensity with concentration for all of the samples. The slopes of these lines all had an $r^2$ value > 0.98 and were not significantly different from each other ($p < 0.05$). This suggests that none of the solvents negatively impacted the fluorescence of the phosphatidylcholine. Interestingly, the phosphatidylcholine showed greatest intensity in EtOH and less intensity in ethyl acetate. This could be due to differences in solubility. Furthermore, this is the opposite trend as was observed in the micrographs and reiterates that the observed differences in the micrographs were due to good solubility of phosphatidylcholine in EtOH and not due to the solvent impacting the fluorescence of the fluorophore.
Figure 5.8 Micrographs of glass beads coated in fluorescent phosphatidylcholine in oil. Micrographs A, C, and E were taken under bright field and B, D, and F were taken using fluorescence mode. Figures A and B show the control sample with no solvent; C and D show the sample treated with EtOH; and E and F show the sample treated with ethyl acetate.
Figure 5.9 Fluorescence intensity at 534 nm of nitrobenzoxadiazole-labelled phosphatidylcholine in various solvents

### 5.3.6 Residual EtOH

The results in Table 5.3 indicate that almost all of the EtOH was removed from solvent substitution chocolate with only 0.02% remaining in the chocolate. The EtOH control had about the same amount of residual EtOH as the sample with EC in EtOH and the control had no EtOH when error is taken into account. Furthermore, the internal standard was nearly fully recovered; it was measured at 0.2032 g/100g but was expected to be slightly higher at 0.2302 g/100g. This difference is likely within experimental error. Perhaps with a longer incubation period any residual EtOH could be eliminated from the chocolate. These results indicate that the observed effects of EtOH were caused by the transient EtOH that has been evaporated rather than EtOH remaining in the samples.

Table 5.3 Concentration of residual EtOH in chocolate samples measured by enzyme assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of EtOH (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal standard</td>
<td>0.2032 ± 0.0084</td>
</tr>
<tr>
<td>Control</td>
<td>0.0011 ± 0.0010</td>
</tr>
<tr>
<td>EtOH control</td>
<td>0.0260 ± 0.0018</td>
</tr>
<tr>
<td>Ethylcellulose in EtOH</td>
<td>0.0218 ± 0.0006</td>
</tr>
</tbody>
</table>
5.4 Conclusions

This work has lead to a better understanding of the mechanism of heat resistance in EC solvent substitution chocolate. Lecithin at the surface of sucrose crystals prevents interactions from occurring between EC and sucrose. This leads to a lower heat resistance than in lecithin-free samples. It was found that the EtOH present in EC solvent substitution chocolate can remove some lecithin phospholipids, particularly phosphatidylcholine, from the sucrose surface by dissolving them. Furthermore, EtOH can dissolve a small portion of the sucrose crystals which makes their surfaces sticky causing aggregation and network formation. Both the removal of phosphatidylcholine and dissolution of sucrose result in increased heat resistance when EtOH is used for manufacture of the samples. In contrast, EtOH seemed to have a negative impact on heat resistance of samples made with skim milk powder, decreasing the heat resistance possibly due to casein destabilization. Cocoa powder was not affected by the presence of EtOH. There was also some evidence that EC may be able to interact with the lactose in skim milk powder and the natural starch present in cocoa powder and this may enhance heat resistance. These results will help to develop better formulations and methods for the production of EC-stabilized heat resistant chocolate.

References

2. US Pat., 2 904 438 (1959)


CHAPTER 6

Engineering the thixotropy of ethylcellulose oleogels by modulation of polymer solubility in triglyceride oils

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Abstract
An ethylcellulose (EC) organogel was engineered with thixotropic properties. EC has been used to form oleogels with vegetable oils as the solvent phase. These gels tend to set at very high temperatures and, once set, can be irreversibly broken by shear forces such as those present during manufacture and use. To overcome this, thixotropic EC oleogels were produced that can recover all of their viscosity after removal of the shear. By changing the solubility parameters of the oil phase, particularly by matching the Hansen hydrogen bonding solubility parameter of the oil phase to that of EC, such as by addition of glycerol monooleate (GMO), the solubility of the EC in the oil can be increased. This increase in solubility leads to the production of fully thixotropic gels with viscosities that can be tailored by altering the EC concentration or viscosity. Furthermore, a gel made with 5-8% EC 10 cP and 40-50% GMO in a variety of oils has favourable texture and water vapour barrier properties which make it a good candidate for use in cosmetics as a replacement for petroleum jelly.

6.1 Introduction
Ethylcellulose (EC) has been identified as a polymer organogelator. The EC molecule is synthesized by reacting cellulose dissolved in an alkaline solution with ethyl chloride. The resulting EC-derivative is a linear polymer of 1,4-β-D-glucose units with ethoxy substitutions at carbons 2, 3, or 6. The degree of substitution must be 2.3-2.6 out of a possible 3 for the EC to be soluble in organic solvents and insoluble in water. This characteristic is necessary for organogelation to occur. The polymers are available in various molecular weights. The molecular weight is characterized by the viscosity in centipoises (cP) of a 5% solution of the polymer in a mix of 80% toluene, 20% ethanol. This study will focus on use of EC polymers with ethoxyl contents of 48-49.5% corresponding to a degree of substitution of approximately 2.5 and which have viscosities of 10, 20, or 45 cP.
EC organogels are formed by heating the EC in oil to above its glass transition temperature\textsuperscript{6} which is 145°C and then allowing the solution to cool during which the gel forms. The EC polymer strands are able to interact with each other via hydrogen bonds to create the gel network.\textsuperscript{7} Furthermore, solvent-solvent and polymer-solvent interactions have also been shown to be responsible for gel properties.\textsuperscript{7}

One main use of EC organogels has been thought to be as a fat replacer.\textsuperscript{8} An organogel with a fatty acid profile very high in unsaturated fatty acids, low in saturated fatty acids, and having zero \textit{trans}-fatty acids could be used to replace fats typically high in saturated and \textit{trans}-fatty acids which could offer health benefits.\textsuperscript{9} Organogels are advantageous compared to liquid oils because they offer functional benefits such as reduced oil migration\textsuperscript{10} and texture profiles which more closely match solid fats.\textsuperscript{8}

It has been observed that the addition of surfactants to the EC organogel system can result in marked changes in the properties of the organogel including altering the appearance and mechanical strength\textsuperscript{6} among other things.\textsuperscript{11} It is thought that the surfactant has the ability to plasticize the EC\textsuperscript{2} and the properties of the surfactant such as the headgroup size would allow different degrees of plasticization.\textsuperscript{12} For example, a surfactant with a small hydrophilic headgroup such as glycerol monooleate (GMO) would have a stronger plasticizing ability than sorbitan monooleate. Therefore, the viscosity of EC oleogels with various surfactants at various concentrations will be studied with the aim of producing a thixotropic oleogel.

Thixotropy is a rheological term that describes a material that thins with time at a fixed shear rate and exhibits an increase in viscosity upon removal of the shear force.\textsuperscript{13} EC gels tend to set at very high temperatures and when subjected to shear after setting the gel irreversibly breaks causing loss of its functionality. This makes it difficult to add EC gels to food or cosmetic products. However, if the gel were thixotropic then processes which require shear after the gel sets would not be as problematic since the gel would be able to recover its viscosity and functionality over time once the shear was removed. The use of surfactants to alter the solubility of EC in oil to produce thixotropic gels will be explored. It is thought that a thixotropic EC oleogel could be used as a solvent- (ethanol) free method of introducing EC into chocolate for the purposes of developing heat resistance in the chocolate. Chocolate making requires shear at
many stages especially during conching and tempering which makes thixotropy a desirable
property in EC gels for use in chocolate. The polymer would be in its extended state in the
thixotropic gel and perhaps this would allow the EC to interact with the sucrose in chocolate in
much the same way as was observed using the solvent substitution method (Chapter 4).
Furthermore, this method may show more organogelation than the solvent substitution method
since the EC would not be preferentially dissolved in the ethanol phase.

Solubility parameters are often used to select the best solvent, or combination of solvents for a
polymer. Hildebrand and Scott\textsuperscript{14} defined the solubility parameter $\delta$ as:
\[
\delta = \left[ (\Delta H_{vap} - RT)/V_m \right]^{1/2}
\]
where $\Delta H_{vap}$ is the enthalpy of vaporization and $V_m$ is the molar volume. Hansen\textsuperscript{15} proposed a
more complex solubility parameter which takes into account the van der Waals forces of the
Hildebrand solubility parameter as well as polar forces and hydrogen bonding. Hansen solubility
parameters (HSPs) are therefore made up of dispersion forces ($\delta_d$), polarity ($\delta_p$), and hydrogen
bonding ($\delta_h$). The sum total of the squares of the three Hansen solubility parameters is equal to
the square of the Hildebrand parameter as shown in Equation 6.2.
\[
\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2
\]
The overarching principle that makes HSPs so valuable is that materials with similar HSPs will
be more likely to dissolve into one another.\textsuperscript{15} HSPs of GMO, oil, and mixtures thereof will be
studied and compared to that of EC to relate experimental results to these solubility theories.

Finally, one particular use of thixotropic gels which will be explored is the development of an
EC oleogel to replace petroleum jelly in the cosmetic industry. Petroleum jelly is derived from
petroleum and is therefore a non-renewable resource. Furthermore, petroleum jelly can be
contaminated with polycyclic aromatic hydrocarbons which are carcinogenic.\textsuperscript{16} For this reason
the European Union has classified petrodatum as a carcinogen and restricts its use in cosmetics.\textsuperscript{16}
Hence, there is a need for a replacement product with similar properties that can be made from
safe, renewable resources.
6.2 Materials and Methods

6.2.1 Gel preparation

ETHOCEL™ standard ethoxy content, premium grade EC polymers with viscosity of 10, 20 or 45 cP were obtained from Dow Chemical Company. Various oils and surfactants were used to make the samples; these ingredients are listed in Table 6.1 along with their sources and abbreviations used throughout the text. The EC powder was mixed with the oil and/or a surfactant. Formulas are described by the concentration of EC in wt% and the ratio of surfactant to oil in the fat phase. For example a formula may be made with 8% EC 10 cP and 40:60 GMO:HOSO. The ingredients were heated to 100-145°C on a hotplate with a stir bar to dissolve the EC. Once fully dissolved the mixture was aliquoted into three glass vials with 10 g of sample and left to set at room temperature.

Table 6.1 Ingredients used and their abbreviations and sources

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Abbreviation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylcellulose</td>
<td>EC</td>
<td>Dow Chemical Company, Midland, MI</td>
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<tr>
<td>High oleic sunflower oil</td>
<td>HOSO</td>
<td>Nealanders International Inc., Mississauga, ON</td>
</tr>
<tr>
<td>Canola oil</td>
<td>-</td>
<td>ACH Food Companies, Inc., Mississauga, ON</td>
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<td>Soybean oil</td>
<td>Soy</td>
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<td>Flax</td>
<td>Omega Nutrition Canada Inc., Vancouver, BC</td>
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<td>Avocado oil</td>
<td>-</td>
<td>Metro Inc., Montréal, QC</td>
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<tr>
<td>High oleic canola oil</td>
<td>HOCO</td>
<td>Nealanders International Inc., Mississauga, ON</td>
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<tr>
<td>High stearic sunflower oil</td>
<td>HSSO</td>
<td>Advanta Ltd., Mar del Plata, Argentina</td>
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<td>Medium chain triglyceride oil</td>
<td>MCT</td>
<td>Stepan Company, Northfield, NJ</td>
</tr>
<tr>
<td>Neobee® 1053</td>
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<td>Stepan Company, Northfield, NJ</td>
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<td>Glycerol monooleate</td>
<td>GMO</td>
<td>HallStar Company, Chicago, IL</td>
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<tr>
<td>Sorbitan monooleate</td>
<td>SMO</td>
<td>Sigma Aldrich, Steinheim, Germany</td>
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<tr>
<td>Triglycerol monooleate</td>
<td>TGMO</td>
<td>Stepan Company, Northfield, NJ</td>
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<tr>
<td>Drewpol® 3-1-O</td>
<td></td>
<td>Stepan Company, Northfield, NJ</td>
</tr>
<tr>
<td>Decaglycerol decanoate</td>
<td>DGDO</td>
<td>Lonza Group Ltd., Allendale, NJ</td>
</tr>
</tbody>
</table>

6.2.2 Viscosity measurement

The viscosities of the gels were measured using an AR 2000 rheometer (TA Instruments, New Castle, DE). After 2 days of storage the samples were sheared by hand in their vials for 15
rotations clockwise and 15 rotations counter clockwise using a flat spatula to assist with transfer onto the base plate of a rheometer. A 60 mm 2° acrylic cone was lowered into the sample to a gap size of 900 μm and the temperature was set to equilibrate to 25°C. A steady state flow test with the shear rate ramping from 20-200 s⁻¹ was used to measure the initial viscosity of the sample. This was followed immediately by a ramp from 200-20 s⁻¹ to give a measure of the immediate viscosity recovery. The sample was then removed from the base plate and placed in a glass vial for storage. The viscosity recovery after one week of storage was tested on the same sample using a ramp from 20-200 s⁻¹. Three replicates were measured for each sample.

The power law model¹³, given in Equation 6.3, was fitted to the data of a shear stress versus shear rate flow curve.

\[ \sigma = k \times \dot{\gamma}^n \]  

6.3

The power law model states that the shear stress (\(\sigma\)) is equal to a consistency coefficient (\(k\)), times the shear rate (\(\dot{\gamma}\)) to the power of \(n\) where \(n\) is the power law index and indicates the type of flow observed. For Newtonian behaviour \(n = 1\); for shear thinning (pseudoplastic) \(n < 1\); and for shear thickening fluids \(n > 1\). The value of \(k\) will be used throughout our analyses as an indicator of viscosity.

### 6.2.3 Oil density measurement

A DE40 density meter (Mettler Toledo, Mississauga, ON) was used to measure the density of the oils and surfactants used in the various formulas. The samples and deionized water were equilibrated to 25°C. The water was used first to perform the daily check. Samples were then tested. The density meter measures density by comparing the resonance frequency of the glass U-tube when filled with air versus water versus the sample during electromagnetic induction. Since air and water have known densities the density of the sample can be calculated from the period of oscillation of vibration when the U-tube is filled with the sample.

### 6.2.4 Hansen solubility parameter calculations

The HSPs for each oil and GMO mixture were calculated using literature values for the HSPs of GMO and sunflower oil. The HSPs of HOSO were not available; however, most vegetable oils
have similar HSPs therefore the HSPs for sunflower oil were used as close approximations. The
densities of the GMO and HOSO were measured and used to convert the wt% of each
component into volume percent since energies of vaporization are calculated on a volumetric
basis. The dispersion parameters for GMO and oil were then multiplied by their respective
volume fraction in the system (Φ) in the GMO-oil mixture and summed to give the δd for the
mixture at each GMO concentration (Equation 6.4).

$$\delta_d^{\text{mix}} = (\Phi_{\text{GMO}} \times \delta_d^{\text{GMO}}) + (\Phi_{\text{oil}} \times \delta_d^{\text{oil}})$$  
Equation 6.4

This was repeated for the δp and δh. The total δ for the mixture was then calculated using
Equation 6.2. The HSPs for the mixtures were then compared to the literature values for HSPs of
ethylcellulose.

6.2.5 Water vapour transmission rate

A gel formula with good viscosity, texture, and full thixotropy was tested for its water vapour
permeability using a method similar to that described by Martini et al. A mixture of 3%
hydroxypropyl methyl cellulose (The Dow Chemical Co., MI, E15), 37.5% w/w of silica gel
(Fisher Scientific, NY, grade 60, 230-400 mesh), 13.2% of a saturated solution of MgCl2·6H2O,
and 46.3% deionized water was prepared. This mixture was measured as having a relative
humidity at 20°C of 96% and was used as the water source for the experiment. AQUALAB
plastic sample cups (Decagon Devices, Inc., WA) were used as sample holders and were filled
with 12 g of the silica gel mixture leaving approximately 2 mm of height to the top of the cup.
The cups were then placed in a freezer at -20°C and left to freeze to ease the spread of sample on
top of the silica gel. A gel sample was prepared with 8% EC 10 cP and GMO and HOSO or
avocado oil at a ratio of 40:60. Control samples were also prepared with HOSO, avocado oil or
petroleum jelly (Vaseline Original Petroleum Jelly, Unilever Canada Inc., Toronto, ON).
Samples were stirred with a metal spatula for 15 rotations clockwise and 15 rotations counter
clockwise and then spread onto the silica filled cups to fill the remaining part of the cup. The
weight of sample added was measured to ensure it was kept relatively constant at 1.6 g. Three
replicates were prepared for each sample including an uncovered sample containing only the
silica gel. The samples were placed in a sealed desiccator with a saturated solution of
MgCl2·6H2O in the bottom to control the humidity at 32.9%. The desiccator was placed in an
incubator at 20°C and the weight change of the samples was measured over time. The water vapour transmission rate (WVTR) was calculated using the following equation:

\[
WVTR = \frac{slope}{A}
\]

where \(slope\) is the slope of the linear portion of the weight loss over time (g/days) plot and \(A\) is the area of the sample which was 12.57 cm\(^2\).

6.3 Results and Discussion

6.3.1 Effect of GMO concentration

The effect of addition of large amounts of surfactant on viscosity of the gels was studied. A typical flow curve result is shown in Figure 6.1. The sample was made with 5% EC 10 cP and GMO:HOSO 45:55. The power law model showed an excellent fit to the shear stress data with an \(R^2 = 0.9998\) and gave a value of \(n = 0.6927\) indicating shear thinning behaviour. All samples tested displayed shear thinning. The value for \(k\), the consistency coefficient, was obtained from the power law fit and plotted against the concentration of GMO in the samples (Figure 6.2). Gels were prepared with 5 or 8% EC 10 cP, HOSO, and GMO at increasing proportions of the oil phase. Samples made with 8% EC had greater viscosity than those made with 5% EC. The maximum viscosity was achieved at 30% GMO at both concentrations of EC; however, this sample displayed only partial thixotropy and showed signs of gel breakage and oil leakage. Gels made with 0-20% GMO appeared to be broken during the pre-shear procedure and had very low viscosity, mainly due to the shear-induced structural breakdown. The greatest viscosity and recovery was achieved between GMO concentrations of 40-50%. The samples made with 40-45% GMO were thus considered as the most promising due to their high viscosity and fully thixotropic character. Subsequent samples were therefore made using these GMO:oil ratios and EC concentration. This sample also had good texture and low oiliness when spread on the skin which is an important attribute for a cosmetic paste. Unfortunately this large proportion of surfactant would likely be extremely detrimental to the heat resistance of a chocolate made with this EC oleogel. The GMO would most likely behave like the lecithin in chocolate and form layers around the sucrose crystals due to their amphiphilic nature. This boundary layer would then impede any interactions between the EC and sucrose and reduce heat resistance similarly to what was observed previously (Chapters 4 and 5). Therefore, these thixotropic formulas were
further studied to better understand the system and their potential for use in cosmetic and food products other than heat resistant chocolate.

Figure 6.1 Appearance (A) and viscosity and shear stress with power law fit (B) of a sample made with 5% EC 10 cP GMO:HOSO 45:55

Figure 6.2 Effect of surfactant concentration on viscosity of gels made with 5% (A) or 8% (B) EC 10 cP, HOSO and GMO
Previous research had shown that addition of small amounts of GMO to EC gels improved the properties of the gel and this was thought to be due to a plasticization effect of the GMO on the EC.\textsuperscript{2} The results observed here warrant further investigation into the effect of addition of large amounts of surfactant on oleogel rheological properties. We initially hypothesized that the GMO was altering the solubilisation of the EC polymer in the oil phase. This can be studied using Hansen solubility parameters. HSPs for the gel components and oil-GMO mixtures are listed in Table 6.2. All three components have similar values for $\delta_d$. The $\delta_p$ of EC is just slightly higher than that of GMO, but much higher than the oil. Therefore, any combination of GMO and oil will not give a $\delta_p$ that matches that of EC. The greater the GMO:oil ratio, the closer its $\delta_p$ value will be to that of EC. Interestingly, the $\delta_h$ of EC lies in-between that of GMO and oil. Therefore, a particular mixture of GMO and oil will have a $\delta_h$ that matches that of EC which is around 9.70 MPa$^{0.5}$. It is clear from the calculated values of HSPs for GMO mixtures that as the concentration of GMO is increased, the $\delta_h$ increases until it reaches 9.65 MPa$^{0.5}$ at a mixture of 45 wt% GMO and 55 wt% oil. This particular combination of GMO and oil gave a superior product with almost full thixotropy, high viscosity, and good texture when applied to the skin. It is thought that at this GMO:oil ratio, the EC has achieved near ideal solubility in the system. As the GMO:oil ratio is increased to 60-70% the total solubility parameter ($\delta$) of the system reaches that of EC. At high GMO ratios there were no marked differences between the gels made with 45-100% GMO. Therefore, we can conclude that hydrogen bonding, and therefore $\delta_h$, in the system plays the major role in determining the gel’s rheological behaviour. Below 45% GMO the EC achieved only partial solubility. Within the range of 20-45% GMO the EC was ideally solubilised for gelation to occur. Partial solubility of the gelator in the solvent is necessary for gelation.\textsuperscript{18} This is why a peak in gel viscosity was observed at 30% GMO. At concentrations less than 20% GMO gels were still formed, however, they were much weaker and the EC was not solubilised enough to produce a thixotropic gel. Thus, the physical properties of EC oleogels display two distinct types of behavior depending on which side of the maximal viscosity-solubility parameter critical region they occupy. In the low surfactant range we encounter solid-gel type behavior, with limited thixotropy, while in the high surfactant range the gels behave more like pastes, with strong thixotropic character.
Table 6.2 Literature and calculated Hansen solubility parameters (in MPa$^{0.5}$) for gel components and combinations thereof

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta_d$</th>
<th>$\delta_p$</th>
<th>$\delta_h$</th>
<th>$\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Literature values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC 10 cP$^{19}$</td>
<td>16.60</td>
<td>8.30</td>
<td>9.70</td>
<td>20.90</td>
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<tr>
<td>Sunflower oil$^{20}$</td>
<td>16.00</td>
<td>1.50</td>
<td>4.70</td>
<td>16.70</td>
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<tr>
<td>GMO$^{21}$</td>
<td>16.10</td>
<td>7.50</td>
<td>15.90</td>
<td>23.80</td>
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<td><strong>Calculated values for oil and GMO mixtures</strong></td>
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</tr>
<tr>
<td>10% GMO$^*$</td>
<td>16.01</td>
<td>2.08</td>
<td>5.79</td>
<td>17.15</td>
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<tr>
<td>20% GMO</td>
<td>16.02</td>
<td>2.67</td>
<td>6.88</td>
<td>17.64</td>
</tr>
<tr>
<td>30% GMO</td>
<td>16.03</td>
<td>3.26</td>
<td>7.98</td>
<td>18.20</td>
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<tr>
<td>40% GMO</td>
<td>16.04</td>
<td>3.85</td>
<td>9.09</td>
<td>18.83</td>
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<tr>
<td><strong>45% GMO</strong></td>
<td>16.04</td>
<td>4.15</td>
<td><strong>9.65</strong></td>
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<tr>
<td>50% GMO</td>
<td>16.05</td>
<td>4.45</td>
<td>10.20</td>
<td>19.53</td>
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<td>60% GMO</td>
<td>16.06</td>
<td>5.05</td>
<td>11.33</td>
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<td>70% GMO</td>
<td>16.07</td>
<td>5.66</td>
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<tr>
<td>80% GMO</td>
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<td>6.27</td>
<td>13.60</td>
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<tr>
<td>90% GMO</td>
<td>16.09</td>
<td>6.88</td>
<td>14.75</td>
<td>22.88</td>
</tr>
</tbody>
</table>

$^*$ wt% of GMO in a mixture of GMO and HOSO

6.3.2 Effect of EC concentration

The effect of EC concentration is shown in Figure 6.3. As the concentration of EC increased the viscosity of the gel also increased. This was expected since the greater the amount of polymer in the system, the more polymer-polymer junction zones can be formed creating a stronger gel network. It was also observed that the proportion of the viscosity that was recovered after one week of storage was close to 100% for concentrations of 5 and 6% but decreased to around 70% for concentrations of 7 and 8% EC. The solvent HSPs would be the same at all of the EC concentrations; therefore, differences in the proportion of recovered viscosity are slightly perplexing. Perhaps, it is related to gelation behaviour rather than solution behaviour. The higher viscosity of the 7 and 8% EC gels may limit the ability of the swollen polymers to diffuse within the solvent and find neighbouring polymers with which to interact. This would limit the gels’ ability to reform all of the tie points that were present in the initial sample resulting in the lower viscosity of the gel after one week of recovery.
6.3.3 Effect of EC molecular weight

The effect of EC molecular weight (expressed as a viscosity in cP) on the viscosity and recovery of the gels is shown in Figure 6.4. There was a clear trend of increasing gel viscosity with increased EC viscosity. This trend was expected since the higher molecular weight EC polymers would swell to a greater size and lead to a greater viscosity in the gel. All of the EC viscosities showed nearly 100% viscosity recovery after one week of storage. The HSPs of EC is very similar at the different viscosities. Therefore, it was expected that these gels show similar behaviour. The gel made with EC 45 cP had a very high viscosity and therefore its texture was very thick which was thought to be less desirable in a cosmetic paste than those gels made with 10 or 20 cP.
6.3.4 Effect of surfactant type

The results have clearly shown that addition of large amounts of GMO to an EC oleogel led to the development of a thixotropic gel. Several other surfactants were studied for their efficacy in producing thixotropic gels. Sorbitan monooleate (SMO), triglycerol monooleate (TGMO), and decaglycerol decaoleate (DGDO) are common food grade surfactants that were mixed with HOSO and EC to produce gels (Figure 6.5). TGMO and SMO both showed much higher initial viscosity than GMO but they recovered only less than 30% of their viscosity after one week. It is thought that these surfactants aren’t as good solvents for EC as GMO. It is likely that the differences observed are due to differences in the Hansen solubility parameters for the various surfactants. SMO has a lower $\delta_h$ than GMO (Table 6.3) at 10.3 MPa$^{0.5}$ compared to 15.9 MPa$^{0.5}$. The solubility parameters for the SMO-oil mixture were calculated using the volume fraction method in Equation 6.4 for the oil phase of the 40:60 SMO:HOSO sample and the experimentally determined density which was 0.9893 g/mL. The $\delta_h$ for the SMO mixture was much lower than that of the GMO mixture. Therefore, the EC was better solubilized in the sample made with GMO leading to the greater proportion of viscosity recovery. The EC was less solubilized in the SMO sample which lead to better gelation of the EC in this sample and the corresponding high viscosity with low viscosity recovery. This is similar to what was observed in the peak region in Figure 6.2. Unfortunately, solubility parameters were not available for TGMO or DGDO. It would be expected that TGMO would have an even lower hydrogen bonding parameter than SMO, and DGDO would have the lowest of them all. Therefore, EC
would have the poorest solubility in DGDO at this surfactant concentration. Adding a greater amount of these surfactants might change the solubility of EC in the oil phase leading to a thixotropic gel. However, that is not ideal since the surfactants would likely have a greater cost than the oil.

![Figure 6.5 Effect of surfactant type on viscosity and recovery of gels made with 8% EC 10 cP and a surfactant:HOSO ratio of 40:60](image)

Table 6.3 Hansen solubility parameters for SMO and its mixture of 40% in oil

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\delta_d)</th>
<th>(\delta_p)</th>
<th>(\delta_h)</th>
<th>(\delta)</th>
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<tr>
<td>Literature values</td>
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<td></td>
</tr>
<tr>
<td><strong>EC 10 cP</strong></td>
<td>16.60</td>
<td>8.30</td>
<td>9.70</td>
<td>20.90</td>
</tr>
<tr>
<td><strong>SMO(^{22})</strong></td>
<td>17.20</td>
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<td>10.30</td>
<td>21.48</td>
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<td>Calculated values for oil and surfactant mixtures</td>
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<td></td>
</tr>
<tr>
<td>40% GMO</td>
<td>16.04</td>
<td>3.85</td>
<td>9.09</td>
<td>18.83</td>
</tr>
<tr>
<td>40% SMO</td>
<td>16.46</td>
<td>3.86</td>
<td>6.83</td>
<td>18.23</td>
</tr>
</tbody>
</table>

6.3.5 Effect of oil type

Several different types of oils were tested for their efficacy in creating thixotropic gels. Medium chain triglyceride (MCT) oil is widely used as cosmetic oil and is made using coconut or palm kernel oils. All of the oils tested showed similar results with only small variations in viscosities and all oils were able to create a thixotropic paste (Figure 6.6). The gel made with MCT oil showed a lower viscosity and an immediate recovery viscosity that was very close to its initial
and recovered viscosities. This oil\textsuperscript{23} has $\delta_h = 4.1 \text{ MPa}^{0.5}$ whereas that of the other oils is around 4.6-4.7 MPa\textsuperscript{0.5}\textsuperscript{20}. However, not all of the oils tested have been characterised for HSPs, so further comparisons cannot be made. The small variations in viscosity observed can also be explained by variations in the densities of the oils tested. HSPs are calculated based on volume fractions however these samples were made based on equal weight proportions of GMO to oil. Therefore, samples with oils of higher density would end up having a slightly lower volume fraction of oil to GMO and therefore a higher $\delta_h$ than oils of lower density since more GMO results in a greater $\delta_h$. The $\delta_h$ for each of the oil mixtures except MCT oil was estimated using the measured densities of the oil and $\delta_h = 4.7 \text{ MPa}^{0.5}$. A plot of initial gel viscosity versus $\delta_h$ (Figure 6.7) shows the expected trend that as the $\delta_h$ approaches that of EC (9.70 MPa\textsuperscript{0.5}) the viscosity decreases indicating a loss of EC gelation due to greater solubilisation of the EC. This mimics the right-hand side of the peak observed in Figure 6.2 depicting the viscosity of the gels with increasing GMO concentration.

![Figure 6.6 Effect of oil type on viscosity and recovery of gels made with 8% EC 10 cP and a GMO:oil ratio of 45:55](image)

132
6.3.6 Comparison to a commercial cosmetic product

6.3.6.1 Water vapour transmission rate

The WVTR of two gels were compared to a commercially available petroleum jelly product in Table 6.4. It was found that gels made with 8% EC 10 cP and 40:60 GMO:oil significantly reduced water vapour transmission compared to an uncovered control. This was the case for gels made with HOSO or avocado oil. Interestingly, the ungelled oil offered the same WVTR as the gelled sample. The gelled sample, however, had the added benefit of high viscosity and a reduced oily feeling on the skin compared to the ungelled sample. The commercial petroleum jelly product almost completely blocked the loss of water. Its WVTR was significantly less than those of the gels. Therefore, the gels have good barrier properties, though petroleum jelly did perform better under these test conditions. It is important to note that petroleum jelly has a melting point near body temperature (37°C) and this would likely affect its barrier properties when used on the skin.
### Table 6.4 Water vapour transmission rate (WVTR) of gel samples and a comparable commercial product*

<table>
<thead>
<tr>
<th>Sample</th>
<th>WVTR g·days⁻¹·cm⁻² (×10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncovered</td>
<td>49 ± 11ansa</td>
</tr>
<tr>
<td>HOSO</td>
<td>2.7 ± 1.5b</td>
</tr>
<tr>
<td>HOSO gel</td>
<td>2.8 ± 0.9b</td>
</tr>
<tr>
<td>Avocado oil</td>
<td>3.8 ± 2.7b</td>
</tr>
<tr>
<td>Avocado gel</td>
<td>3.5 ± 2.0b</td>
</tr>
<tr>
<td>Petroleum jelly</td>
<td>0.035 ± 0.0086c</td>
</tr>
</tbody>
</table>

*Values with the same superscript are not significantly different (p < 0.05).

#### 6.3.6.2 Viscosity

The viscosity of a fully thixotropic EC gel can be tailored by modifying the concentration or viscosity of the EC. A formula with 5-8% EC, EC 10-45 cP, 45:55 GMO:HOSO can have an initial viscosity ranging from 3.4-99.4 Pa·s. Adjusting the EC concentration beyond the 5-8% interval would likely increase this viscosity range even further. The commercial petroleum jelly had initial, immediate, and recovered viscosities of 200.4 ± 4.9, 15.1 ± 2.9, and 168 ± 37 Pa·s at 25°C. This product clearly had greater viscosity than any of the EC gel formulas tested and also showed full thixotropy. Again, the melting temperature of petroleum jelly being close to body temperature would greatly change (lower) its viscosity when spread on the skin. This would not occur with the EC gels.

#### 6.4 Conclusions

The thixotropy of EC oleogels was successfully engineered by modulation of polymer solubility in triglyceride oils. A gel can achieve 0-100% viscosity recovery after shearing depending on how closely the hydrogen bonding solubility parameter of the solvent phase matches that of the EC polymer. Full viscosity recovery was achieved with GMO at concentrations of 40-50%. Gels showing full viscosity recovery show great potential for use as fat replacers in food products and as cosmetic ingredients especially in those products that would introduce shear at temperatures where the gel has already set. Gels have been developed which have favourable textural and water vapour barrier properties for use on the skin.
References


OVERALL CONCLUSIONS AND FUTURE WORK

This work has produced a novel method of manufacturing heat resistant chocolate. Previous strategies were analysed to try to understand how heat resistance had been achieved in the past. This led to the conclusion that most successful strategies focused on creating a sucrose network within the chocolate that could hold the shape and resist deformation of the chocolate at temperatures above the normal melting temperature. We developed a method of manufacturing chocolate by using ethylcellulose (EC) as a functional ingredient that was capable of providing heat resistance to chocolate.

Our hypothesis could be accepted since it was found that EC was easily incorporated into conventional chocolate by first dissolving the EC in ethanol (EtOH), then mixing with the chocolate, and thereafter evaporating the EtOH. The resulting chocolate had significant heat resistance that could be optimized by altering the ratio of EC to EtOH in solution, and the amount of EC added. The chocolate formulation also had a great impact on the heat resistance. This new method for adding EC to food products has some advantages over having to heat the EC to above its glass transition temperature. The fat phase does not need to be heated and therefore problems with oxidation would be avoided. Unfortunately the EtOH can be dangerous to work with, and may be a deterrent to some consumers who keep away from EtOH. Furthermore, the EtOH may be quite costly to use though incorporating an EtOH recovery step would reduce this cost.

The mechanism of heat resistance in this chocolate was explored such that future method and formulation optimization techniques could be judiciously selected. Large deformation mechanical testing on model systems showed that only a minor portion of the heat resistance was due to organogelation of the fat phase of the chocolate. Therefore, our first hypothesis that heat resistant chocolate could be made by gelling the fat phase of the chocolate using EC was rejected. The EC could be dissolved in a solvent or by the heat method in the fat phase and added to the components of chocolate and results showed that gelation could only account for a fraction of the hardness observed.

Instead, it was shown that EC interacting with other components in the chocolate produced the majority of the heat resistance. Atomic scale molecular dynamics simulations were used as a tool
to predict if EC could interact with sucrose in a hydrophobic environment. This technique along with Fourier-transform infrared spectroscopy showed that EC and sucrose can form hydrogen bonds with each other. These experiments showed that molecular dynamics simulations are a great tool that can be used to study food systems at the molecular level. Furthermore, the results corresponded very well with multiple techniques studying physical samples. Mechanical testing and scanning electron microscopy showed that the interaction between EC and sucrose was responsible for the structure formation within the chocolate that produced the observed heat resistance. These results confirmed our hypothesis that in solvent substitution chocolate EC and sucrose are able to hydrogen bond to one another leading to structure formation that provides heat resistance in the chocolate. Network formation was also found in samples made with EC and glucose or native starch suggesting that the hydrogen bonding observed was unique to EC polar groups bonding to free hydroxyl groups on carbon two of a glucose moiety in a crystalline particle. This result and mechanical testing with other chocolate ingredients have indicated a likely interaction between EC and the lactose present in skim milk powder as well as EC and the natural starch present in cocoa solids. Therefore, EC can likely be used in many different food systems rich in sugars and starches to produce a network structure. Foods like sweetened nut butters, confections, cream fillings, and cookies may exhibit improved texture, oil binding, or shelf life through the addition of EC.

Fourier-transform infrared spectroscopy and mechanical testing were also used to study the impact of lecithin at the surface of the sucrose in heat resistant chocolate. It was found that lecithin created a physical boundary at the surface of the sucrose which impeded the ability of EC to interact with the sucrose resulting in a negative effect on heat resistance. However, several techniques revealed that the EtOH used to dissolve the EC in solvent substitution chocolate was able to dissolve some of the lecithin phospholipids from the surface of the sucrose. The sucrose was then free to interact with EC resulting in a positive impact on heat resistance. Again, this confirmed the hypothesis that EtOH is able to remove some of the phospholipids, namely PC, from the sugar surface, allowing the surface of the sugar to be available for interaction with EC. This result is especially important to the manufacture of heat resistant chocolate since most chocolates are made with emulsifiers. Removing emulsifiers from the chocolate formula would likely increase the viscosity of the chocolate and may cause textural changes to the molten
chocolate in the mouth. However, these changes may be acceptable to produce a superior heat resistant chocolate.

Interestingly, it was found that addition of EtOH itself, without any EC, to chocolate could enhance heat resistance by dissolving a small portion of the sucrose crystals causing aggregation and network formation of the solid particles. This result was surprising considering the same is seen when water is added to chocolate but sucrose is 1000 times less soluble in EtOH than water. Furthermore, this low solubility of sucrose in EtOH actually delayed the onset of particle aggregation in the chocolate which is necessary to be able to easily mold or enrobe articles with the chocolate. Many of the patents reviewed focused on methods that delayed the onset of particle aggregation and it led to the development of some very complicated, time consuming, and equipment intensive methods. The use of a volatile solvent with low sucrose solubility would be a very easy method to produce heat resistance in chocolate. However, the above mentioned concerns with the use of EtOH would also apply to other solvents.

Although the solvent substitution technique proved very successful at producing heat resistant chocolate it was thought that the use of EtOH in chocolate was not ideal due to safety and cultural concerns. Therefore, it was hypothesized that a thixotropic EC oleogel could be developed by highly plasticizing the EC through addition of surfactants. Thixotropy would allow the gel to be incorporated into chocolate without the gel irreversibly breaking and without the need of EtOH. It was found that EC gels could be formulated to have thixotropy by modification of the polymer solubility in the oil phase. Gels had variable thixotropy depending on how similar the hydrogen bonding solubility parameter of the oil phase matched that of EC. This was demonstrated by adding glycerol monooleate (GMO) to vegetable oil for use as the solvent phase. At 40-50% GMO in the oil phase the hydrogen bonding solubility parameter of the solvent matched that of EC and full thixotropy was observed. These gels have great potential for use as fat replacers in food products as well as in cosmetics to replace potentially harmful ingredients such as petroleum jelly. Unfortunately, the high amount of GMO made these gels unsuitable for use in heat resistance chocolate.

The heat resistant chocolate and thixotropic gels developed are just two examples of novel ways to use EC. The insights into the mechanism of heat resistance gained through this research will
allow for chocolate formulations to be optimized for heat resistance. For example, it would be ideal to create chocolate without any emulsifiers such that all of the sucrose would be available to interact with EC. Furthermore, the concentration of EtOH could be optimized such that the EC is ideally solubilized and the amount of EtOH present positively impacts heat resistance. Future work concerning the commercialization of solvent substitution chocolate should also focus on improving the methods to remove EtOH and to develop an EtOH recovery step. Finally, various other food systems that could benefit from addition of EC using the solvent substitution method or thixotropic gels should be explored.