Comparing the taste-modifying properties of nanocellulose and carboxymethyl cellulose

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Abstract: The taste-modifying properties of nanofibrillar cellulose (NFC) and carboxymethyl cellulose (CMC) are compared for the first time. The samples were prepared in the form of gels, with and without added sweet and bitter taste components. As viscosity itself is known to affect taste perception, the viscosities of NFC and CMC samples were set to the same level as shear rates commonly found in the oral cavity. A trained panel of 10 assessors evaluated the bitterness and sweetness of the samples. Further, the assessors were given an opportunity to describe the samples in free words. The taste-modifying capacities of the thickening agents were at the same level when sweet compounds were added. However, CMC was better able to reduce the bitterness of quinine hydrochloride than NFC, which did not show any bitterness-reduction ability with the compound. This was unexpected, as our previous studies of NFC showed fairly high binding capacity with quinine. The open-ended responses revealed that the NFC-containing samples had an astringent sensation, while certain assessors observed a sensation of saltiness in the CMC samples. This may explain the inability of NFC to mask the bitterness of quinine hydrochloride, as astringency may act as a bitterness enhancer, while saltiness may suppress it. Both thickening agents were perceived as slightly bitter. Our study reveals the need for further assessment of the orosensory properties of NFC, particularly the magnitude and origin of its astringency, before it can be fully utilized in food industry applications.

KEYWORDS
nanocellulose, taste modification, bitterness, sweetness

1 | INTRODUCTION

Of the taste modalities, bitterness in particular is often described as unpleasant. The bitterness has therefore been reduced by many methods, involving the removal of bitter-tasting compounds or adding other flavors to suppress or mask the bitterness, the use of physical barriers including encapsulations, coatings, or emulsions, and the use of bitterness-inhibiting compounds (Gaudette & Pickering, 2013; Ley, 2008). Bitterness reduction is an important question for both pharmaceutical and food development, but the objectives are different: The development of pharmaceuticals aims to reach a palatable/pleasant level of bitterness, while the objective of food development is to modify the overall sensory profile to be desirable for consumers (Gaudette & Pickering, 2013).
Using physical barriers to reduce bitter compounds from coming into contact with taste receptors by restricting their release of them during in-mouth processing or by inhibiting their interactions with receptors is one possible approach to suppress bitter sensations originating from food (Sun-Waterhouse & Wadhwa, 2013). Increasing the viscosity is one approach used when utilizing physical barriers. Numerous studies have reported the effect of increased viscosity on bitterness. Moskowitz and Arabe (1970) studied the effect of viscosity on perceived taste intensity using various concentrations of carboxymethyl cellulose (CMC) and taste compounds including glucose, citric acid, sodium chloride, and quinine sulfate. The increase in viscosity caused a decrease in the taste intensities and even in the nondetectable taste with bitter quinine sulfate at low concentrations (Moskowitz & Arabe, 1970). Pangborn et al. (1973) investigated the capacity of CMC (at low and medium viscosities) alongside with other hydrocolloids, such as hydroxypropyl cellulose, sodium alginate, and xanthan, to decrease the bitterness intensity of caffeine. The decrease in the intensity of bitterness was achieved with CMC at low viscosity, and with sodium alginate and xanthan, while CMC at medium viscosity or hydroxypropyl cellulose did not have similar effects. This proposes that the nature of the used hydrocolloid has an effect on its bitterness suppression capability (Pangborn et al., 1973). Further, in a study by Smith et al. (1996), the bitterness intensity of grape-seed tannins was not affected by the increase in CMC concentration at medium viscosity, whereas their astrignency was.

New viscosity-modifying cellulose materials are emerging that may alter taste in the same way as CMCs do. A family of nanocellulose materials in particular has recently attracted a great deal of attention. They are materials with nanoscale widths produced either with chemical, enzymatic, or physical methods from cellulose fibers (Klemm et al., 2011). These materials have large surface areas and surface functionality and thus they are easily chemically modified. Furthermore, they have suitable rheological behavior for the stabilizing and emulsifying applications in the food industry (Gómez et al., 2016; Lee et al., 2017). Particularly for the purposes of food packaging, dense network and thus good mechanical and barrier properties and transparency are advantageous properties (Lee et al., 2017). Based on present knowledge, nanocellulose materials have no or low toxicity (Gómez et al., 2016; Lee et al., 2017). However, further research is needed in this area (Gómez et al., 2016; Lee et al., 2017).

The markets for nanocellulose materials as food additives are still narrow. Although previous problems concerning the cost of the manufacturing processes have been overcome, Gómez et al. (2016) note more research is needed on the behavior of nanocellulose materials in complex matrices such as food. Thus, there is no enough evidence concerning the safety of nanocellulose materials to progress with their wider utilization in commercial food products. Further, to our knowledge there are no systematical studies involving several taste compounds regarding the possible effects of nanocellulose materials on the taste of food. A study by Agarwal et al. (2018) found that softwood cellulotic fiber prolongs the salty taste of the samples. In a study by Golchoobi et al. (2016), no taste changes were found in mayonnaise samples with added nanofibrillar cellulose (NFC) when using hedonic scale from 1 (very poor) to 5 (great).

In our recent study, we revealed that NFC interacts with certain taste compounds such as quinine, caffeine, stevioside, and naringin (Manninen et al., 2020). Measured binding constants varied from 70 M$^{-1}$ for caffeine to 14300 M$^{-1}$ for quinine. These interactions are in the same order of magnitude as seen between quinine and L-lysine and L-arginine, which were able to suppress the bitterness of quinine according to sensory studies (Zhang et al., 2016). However, as we only performed chemical analyses in our previous study, it remains unclear whether the interactions we observed, particularly with quinine, would actually play a role in taste perception and whether they are strong enough to cause the suppression of bitterness and sweetness. In this study, we therefore aimed to evaluate whether these interactions between NFC and taste compounds are strong enough to change the intensity of the studied taste modalities. We tested our hypothesis of the taste-modifying properties of NFC by comparing these properties with CMC, which is commonly used as a thickening agent and have been demonstrated to have some abilities to reduce bitterness.

## 2 MATERIALS AND METHODS

### 2.1 Materials

NFC samples were purchased from UMP Biomedicals (Finland). The used material was 1.5 wt% hydrogel (Growdex®). Food-grade CMC sodium salt (Cekol® 4000) came from CP Kelco (Finland). The studied taste compounds were quinine hydrochloride dihydrate (QHCl, Sigma Aldrich, St. Louis, USA), steviol glycoside by Govinda Natur (Neuhofen, Germany), caffeine (Sigma Aldrich), and sucrose Alfa Aesar 99% by ThermoFisher (Kandell, Germany).

### 2.2 Viscosity measurements

The viscosities of the NFC and CMC samples were measured with Rheometer Physica MCR301 using the RHEO-PLUS application. A viscosity of 0.5 wt% NFC prepared
TABLE 1 The prepared references and test samples for the sensory evaluations

| Name       | Thickening agent | Reference | The final conc. of taste compounds (wt%) |
|------------|------------------|-----------|----------------------------------------|
| Sweetness 1| Water            | Sucrose   | 2                                      |
| Sweetness 2| Water            | Sucrose   | 4                                      |
| Bitterness | Water            | QHCl      | 0.0005                                 |
| Number     | sample           |           |                                        |
| 1.         | NFC              | None      | –                                      |
| 2.         | CMC              | None      | –                                      |
| 3.         | NFC              | Sucrose   | 4                                      |
| 4.         | CMC              | Sucrose   | 4                                      |
| 5.         | NFC              | QHCl      | 0.0005                                 |
| 6.         | CMC              | QHCl      | 0.0005                                 |
| 7.         | NFC              | Caffeine  | 0.04                                   |
| 8.         | CMC              | Caffeine  | 0.04                                   |
| 9.         | NFC              | Steviol glycoside | 0.008   |
| 10.        | CMC              | Steviol glycoside | 0.008   |

Figure 1 The results from the viscosity measurements of shear rates (γ, 1/s) and viscosities (η, Pa•s) in logarithmic scale. NFC 0.5% is marked with circles, CMC 0.4% with diamonds, 0.5% with triangles and 0.6% with squares.

from 1.5 wt% stock by carefully mixing it with Milli-Q (MQ)-water was measured three times. The NFC concentration was chosen so that clear thickening was seen in the samples, but the concentration level was kept as low as possible to minimize the exposure of the assessors to NFC. We compared three CMC sample concentrations, ranging between 0.4 and 0.6 wt%, with the NFC. The samples were prepared by weighting a required amount of solid CMC with MQ-water, and carefully mixing to ensure that the samples were uniform and did not contain any bubbles. In the literature, shear rates of 50 1/s and between 10 1/s and 1000 1/s depending on the flow characteristics of the food have been reported for foods in the oral cavity (Shama & Sherman, 1973; Wood, 1968). As the exact shear rate of samples in the mouth was unknown, we inspected the region between 10 and 1000 1/s. In this area, the average viscosity of the NFC samples in the 0.5 wt% concentration was between the viscosities of the CMC samples in the concentrations of 0.5 wt% and 0.6 wt%. However, the viscosity differences measured for the CMC concentrations were approximately in the same order of magnitude as the differences between the NFC replicates. For our study, we therefore chose the same weight percentage of 0.5 wt% for both CMC and NFC. The results from the viscosity measurements in log-scale can be seen as a graph in Figure 1.

2.3 Sample preparation for sensory evaluation

The samples containing taste compounds and a thickening agent, either NFC or CMC, were prepared by mixing a required amount of taste stock solution with CMC or NFC to reach a final concentration of 0.5 wt% of the thickening agents and selected final concentrations of the taste compounds, as indicated in Table 1. All samples were mixed carefully for at least 30 s to form uniform samples. The stock solutions for taste compounds were used to prepare both the samples and references. Stock solution concentrations were 8 wt% for sucrose, 0.1 wt% for caffeine, 0.005 wt% for QHCl, and 0.08 wt% for stevioside. The weighted amount of taste compounds was dissolved into weighted amount of active carbon-filtered water to reach the concentrations wanted. The solutions were carefully shaken, and the homogeneity of the solutions were ensured before sample or reference preparation. The reference samples were prepared by diluting weighted amount of sucrose and QHCl stock solutions with weighted amount of water to reach final concentrations indicated in Table 1. All the samples were presented to the assessors at room temperature.
The sensory evaluation was performed in the ISO 8589 standardized sensory laboratory of the Functional Foods Forum (University of Turku). Altogether 10 voluntary assessors (nine females, one male, working-age adults) participated in the study, all with backgrounds in sensory profiling via various projects and known abilities to identify and rank taste compounds. The ethical statement for the study was applied from the University of Turku Ethics committee (statement 56/2019) and informed consent was obtained from each subject prior to their participation in the study.

Training for the analysis consisted of two training sessions lasting approximately 1 hr each. In the first session, the panelists were asked to evaluate the bitterness and sweetness of five randomly chosen samples out of a set of 10 samples presented in Table 1 (except for caffeine) using a line scale (0 to 10) and to provide additional remarks about them. The latter five samples were introduced and assessed during the second training session. After the trainings, we increased the caffeine levels from the original 0.004 to 0.005 wt% used during training to enhance its perception. Furthermore, the reference samples and their intensities were agreed upon in the training sessions.

2.5 | Sensory evaluation procedure

The samples were weighed on plastic spoons to reach a weight of 3.5 g per sample. The spoons were placed against the side of a tray to help the assessors lift them. Approximately 40 mL of each reference taste solution was served with the samples. To minimize the possible effects of the slight color differences in the samples, the samples were presented to the assessors on white plastic spoons and red lighting was used during the evaluation sessions. The placement of the spoons and references on the tray are presented in Figure 2. The samples were expectorated after tasting. Wheat crackers and water were served with the samples and the assessors used them to clean their palates. The assessors were guided to ask for a replacing sample if needed.

The assessors were asked to rate the intensity of the bitterness and sweetness of each sample using a continuous line scale from 0 (no sensation at all) to 10 (extremely strong). Participants were additionally instructed to describe the samples with extra comments when needed. The sweetness intensities of reference samples with either 2 wt% or 4 wt% of sucrose (sweetness 1 and sweetness 2 in Table 1) were agreed to have sweetness values 4 and 8 in trainings. The bitterness intensity of reference sample containing QHCl in the concentration of 0.0005 wt% was agreed to have bitterness value of 5. All samples were evaluated as triplicates in different sessions. Compusense Cloud (Compusense, Inc., Guelph, ON, Canada) was used for data collection in the sensory laboratory.

2.6 | Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 25 (IBM, Armonk, NY, USA). The normality of the results variances of for each sample was tested with Shapiro-Wilk ($p < 0.05$). As most of the variances were nonnormally distributed and further, the data were nonindependent, a nonparametric Wilcoxon signed-rank test was performed to evaluate the statistical differences between the samples containing the same taste compound addition but different thickening agent (NFC or CMC). Each pair of thickening agents with a different taste compound were studied separately. Further, all the samples with different taste compound additions and/or thickening agents were compared using the Friedman test and Wilcoxon tests with Bonferroni corrections. The criterion for statistical significance in all the tests was $p < 0.05$.

Differences among sample ratings in bitterness and sweetness were analyzed by applying a two-way mixed factorial ANOVA model for all the samples (fixed factors), sessions and assessors (random factors) with the sample effect and session $\times$ assessor and sample $\times$ session interactions. The Tukey’s HSD post hoc test was used to identify between-group differences. In addition, Friedman and Wilcoxon tests with Bonferroni corrections ($p < 0.05$) in the case of nonnormally distributed variances were applied. This was done by either comparing the scores given to each sample in the three evaluation replicates (reproducibility) or the scores given to each sample by different assessors (consensus).
3 | RESULTS AND DISCUSSION

3.1 | Panel reproducibility and consensus

We evaluated panel reproducibility and consensus using two-way ANOVA and post hoc tests or the Friedman and Wilcoxon tests, as described in detail previously. Based on the two-way mixed factorial ANOVA model samples were different, but the sessions were not. Moreover, the interactions sample x session for the bitterness (F18,162 = 1.110) and sweetness (F18,162 = 0.509) as well as session x assessor for the bitterness (F18,162 = 1.357) and sweetness (F18,162 = 1.508) were all nonsignificant (p > 0.05). Significant assessor effects were present for both bitterness and sweetness. The differences between assessors were expected as there are variations for the sensitivity for taste perceptions as shown with caffeine and sucrose in Puputti et al. (2018).

With non-normally distributed data, we observed no significant differences between the assessors or triplicate sensory evaluation sessions according to the Friedman and Wilcoxon tests with Bonferroni corrections for bitterness and sweetness. The only statistical difference we found was between the intensity of perceived bitterness in the different evaluation sessions of the pure CMC samples (Z = 2.527, p = 0.036), which had relatively low level of bitterness.

3.2 | Statistical differences between the samples

The mean values of the samples’ sweetness and bitterness are presented in Table 2 with standard deviations. We found no statistical difference (p < 0.05) between the intensity of bitterness of pure thickening agents and samples with added sucrose or steviol glycoside. The average bitterness of the CMC and NFC thickening agents without added taste compounds were evaluated to be 1.97 ± 1.87 and 1.65 ± 1.71, respectively, indicating slight bitterness. This is surprising, as to our knowledge, no prior studies have indicated the bitterness of CMC or NFC. However, as bitterness is low, it is likely suppressed in actual food applications by other flavors present in the product. The samples with added sucrose or steviol glycoside were perceived as less bitter, with the mean values being lower than with corresponding pure thickening agents. However, according to the Wilcoxon signed-rank tests, these differences in bitterness were statistically significant (Z = -3.943, p = 0.000) only in pure NFC versus NFC with added sucrose. The bitterness of NFC and CMC thickening agents with added bitter compounds were statistically significantly (p < 0.05) more pronounced than with pure thickening agents, as expected. CMC with bitter taste compounds were evaluated as less bitter than NFC for both added bitter taste compounds (QHCl and caffeine). We found NFC samples with added QHCl (6.17 ± 2.08) to be statistically more bitter (Z = -4.032, p = 0.000) than CMC (3.73 ± 2.12) with the same addition. With caffeine addition, NFC (5.88 ± 2.32) was perceived as more bitter (Z = -2.369, p = 0.018) than CMC (4.35 ± 2.70), but this difference was not statistically significant when Bonferroni corrections were considered. Based on these results, it seems that the effect of the thickening agents on the bitterness reduction is stimuli depended as statistically significant differences were found with QHCl while the differences were not significant with caffeine. One possible explanation could be differences in the possible chemical interactions between the CMC and

### Table 2

| Sample | Thickening agent | Taste compound     | Bitterness | Sweetness |
|--------|------------------|--------------------|------------|-----------|
|        |                  |                    | Mean       | SD        | Mean       | SD        |
| 1.     | NFC              | None               | 1.65       | 1.71      | 0.15       | 0.29      |
| 2.     | CMC              |                    | 1.97       | 1.87      | 0.48       | 0.78      |
| 3.     | NFC              | Sucrose            | 0.31       | 0.62      | 6.74       | 1.46      |
| 4.     | CMC              |                    | 0.40       | 1.23      | 6.46       | 1.76      |
| 5.     | NFC              | QHCl               | 6.17***    | 2.08      | 0.04       | 0.10      |
| 6.     | CMC              |                    | 3.73***    | 2.12      | 0.54       | 1.03      |
| 7.     | NFC              | Caffeine           | 5.88       | 2.32      | 0.03       | 0.08      |
| 8.     | CMC              |                    | 4.35       | 2.70      | 0.18       | 0.47      |
| 9.     | NFC              | Steviol glycoside  | 1.22       | 1.37      | 3.85       | 1.60      |
| 10.    | CMC              |                    | 1.21       | 2.03      | 4.46       | 1.68      |
studied compounds. For example, fluorescence indicator method used prior to study the interactions between NFC and taste compounds (Manninen et al., 2020) could provide useful information to evaluate this possibility. It is possible that CMC may have a better antagonist capability for TAS2R bitter taste receptors than NFC, but this should be further studied. Further, it has to be noted that the changes in concentrations needed to cause noticeable differences in taste perceptions are different with each compound.

Compared to the reference sample, with the same final QHCl concentration in water with a set bitterness intensity of 5, the NFC samples with added QHCl were evaluated as more bitter, whereas the CMC samples with added QHCl were evaluated as less bitter. This suggests that CMC addition decreases the bitterness intensity more significantly than NFC, addition of which seems to even increase bitterness intensity. The lower ability of nanocellulose to suppress bitterness was not expected as our hypothesis was that NFC could have a pronounced effect on the taste of bitter compound quinine. The hypothesis was based on our previous study (Manninen et al., 2020), where we showed that the binding of some compounds to nanocellulose material may be fairly significant, especially in the case of quinine, whose binding constant was measured to be 14300 M$^{-1}$. One possible explanation for the observed behavior may be that the interactions seen in fluorescence indicator studies (Manninen et al., 2020) are simply too small to have an effect on taste and thus to be noticed in sensory studies. Furthermore, the required concentration for bitter compounds, such as QHCl, to be recognized is lower compared to the concentration required for many sweet compounds such as sucrose (Chang et al., 2006). Quinine hydrochloride activates taste receptors even at low concentrations, and the concentration used in this study is high apparently enough to prevent QHCl from fully binding to NFC.

The average sweetness of pure CMC and NFC were evaluated to be 0.48 ± 0.78 and 0.15 ± 0.29, respectively, which are negatable perceived intensities. Further, adding bitter compounds did not change this perception but adding sweet compounds increased the sweetness, as expected. According to the Wilcoxon signed-rank test for comparing the pairs with different thickening agents, pure CMC was perceived as sweeter than pure NFC ($Z = −2.232$, $p = 0.026$). However, with Bonferroni corrections, there are no significant differences between CMC and NFC. No statistical differences were found between CMC and NFC thickening agents with added sweet-tasting compounds. CMC (0.54 ± 1.03) was assessed as slightly sweeter ($Z = −2.281$, $p = 0.005$) than NFC (0.04 ± 0.10) with QHCl addition, but with Bonferroni correction however, the difference was statistically insignificant. The difference may at least partly be explained by the differences in bitterness of pure thickening agents, as the perceived bitterness may suppress the very slight sweetness that the pure thickening agents have. However, the sweetness level was very low and close to 0 (no sensation at all). Sucrose samples with CMC (6.46 ± 1.76) or NFC (6.74 ± 1.46) thickening agents were evaluated as less sweet than the reference samples at a set value of 8, with the same sucrose concentration diluted with water. However, the standard deviations for the assessments were high and thus the set sweetness of the reference sample fit within these margins.

### 3.3 Open-ended question responses

The responses to open-ended questions were focused mainly on chemesthesia/taste or on texture. The main results of the chemesthesia or taste-related answers are presented in Table 3.

Astringency was the most frequent description in the open-ended responses especially for NFC-containing samples. This description was repeated multiple times for all NFC containing samples. It was mentioned in approximately 45% of the 150 open-ended responses concerning NFC-containing samples (only the most common descriptions are shown in Table 3), while the term occurred in only 8% of all the responses describing CMC-containing samples. Eight out of ten assessors perceived astringency in at least one sample, and six out of ten mentioned it at least once in all the NFC-containing samples. A tingly or burning sensation was another common description, which was found in both NFC (7%) and CMC (15%). A cooling sensation (NFC 7%, CMC 13%) and saltiness (NFC 1%, CMC 17%) were also observed in many samples. These descriptions were all more common for CMC than NFC, except for the cooling sensation. Salty taste in particular was associated almost exclusively with the CMC samples.

The literature shows that astringent phenolic compounds, such as flavanol-3-glycoside, contribute to the bitterness of caffeine in tea samples (Scharbert & Hofmann, 2005). Further, saltiness is a known suppressant of many tastes, such as bitterness (Keast et al., 2001). Thus, we expect that both NFC astringency and potentially also CMC saltiness have an impact on the perceived bitterness of the samples. The cause of NFC astringency is currently unclear because, to our knowledge, cellulose chains have not been shown to cause perception of astringency. However, other effects, such as small particle size or the possible remnants of native cellulose, may cause astringency.

We were unable to make any clear conclusions concerning the descriptions related to the texture of the samples, indicating that sample textures differed only slightly from each other. Certain descriptions, including for example...
thickening, were found from open-ended question responds of all samples but were repeated only one to three times per sample. Further, certain samples were described with contradictory terms for the texture as for example NFC + QHCl samples were described as both ‘thick’ and as ‘runny’. As the viscosity of the sample materials was difficult to match due to the differences in the properties of the thickening agents, the small differences in perceived viscosity may affect the taste. However, the open-ended questions did not reveal major differences in the perceived textures of the samples.

4 CONCLUSION

The taste-modification properties of NFC and CMC were compared for the first time by evaluating the bitterness and sweetness of pure NFC and CMC and with added taste compounds. As our study is one of the first to study both taste-modification properties and the taste qualities of NFC, we provide important information of the possibilities of utilizing NFC in food applications.

In contrast to what we expected based on our previous studies of NFC interactions with taste compounds, NFC does not act as a more effective bitterness modifier than CMC, which was used as a reference. CMC with added QHCl was perceived as less bitter than NFC with the same addition. With added QHCl, NFC bitterness was even more pronounced than the set bitterness of used reference (QHCl in water) at the same final concentration. On the contrary, assessors evaluated the samples with added sucrose and thickening agents as less sweet than the reference samples containing the same concentration of sucrose in water. However, as the comparisons were made between set references and perceived intensities of taste qualities, we could not perform statistical tests. Further, large standard deviations suggest that the differences in these cases may be statistically insignificant. Interestingly, both thickening agents studied here were perceived as slightly bitter. To our knowledge this has not been reported before.

The open-ended responses revealed that NFC in particular was perceived as astringent. The astringency found in the NFC samples may be one explanation for the inability of NFC to reduce the bitterness of the samples and for the increased bitterness of QHCl when added to NFC. As astringency was not an expected characteristic, its magnitude and cause were not studied but should be in later studies. Furthermore, the same study should be conducted with several concentrations of taste compounds, particularly QHCl and thickening agents, to better understand the underlying interactions.
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AUTHOR CONTRIBUTIONS
H. Manninen designed the study, collected the data, and interpreted the results as well as drafted the article. M. Sandell designed the study and drafted the article. S. Mattila designed the study and participated in the data collection as well as drafted the article. A. Hopia and T. Laaksonen designed the study and participated in the drafting of the article.

CONFLICTS OF INTEREST
Authors declare no conflicts of interest.

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