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Bruna Tischer

DESENVOLVIMENTO DE MÉTODOS ANALÍTICOS ENVOLVENDO ENTALPIMETRIA NO INFRAVERMELHO

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2017

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutor em Ciência e Tecnologia dos Alimentos.**

Orientador: Prof. Dr. Juliano Smanioto Barin
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Juliano Smanioto Barin, Dr.
(Presidente/Orientador)

Adilson Ben da Costa, Dr. (UNISC)

Cristiane Franco Codevilla, Dra. (UFRGS)

Fábio Andrei Duarte, Dr. (UFSM)

Milene Teixeira Barcia, Dra. (UFSM)

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RESUMO

DESENVOLVIMENTO DE MÉTODOS ANALÍTICOS ENVOLVENDO ENTALPIMETRIA NO INFRAVERMELHO

AUTORA: Bruna Tischer
ORIENTADOR: Juliano Smanioto Barin

O controle de qualidade de alimentos e de medicamentos é feito em grande parte utilizando métodos recomendados nos compêndios oficiais, entre eles a titulação e a gravimetria, os quais envolvem vários aspectos negativos e em desacordo com os princípios da química analítica verde. Métodos entalpimétricos se baseiam na determinação da entalpia das reações químicas. Na análise entalpimétrica é efetuada a determinação quantitativa de um reagente ou catalisador através da variação de entalpia de uma reação química, que pode ser medida direta ou indiretamente. As principais abordagens para a análise entalpimétrica são a titulação termométrica, entalpimetria por injeção direta (DIE, do inglês *Direct Inject Entalpimetry*) e entalpimetria em fluxo. No entanto, apesar de hoje em dia haver a disponibilidade de instrumentos comerciais para este fim, essas técnicas foram substituídas e quase desapareceram da literatura, provavelmente devido ao custo elevado de dispositivos de microcalorimetria e pela limitação da baixa frequência de análise, levando à escolha de outros métodos analíticos. Atualmente, existe a necessidade do desenvolvimento de métodos analíticos que forneçam resultados rápidos, com baixo custo, pequeno consumo de reagentes e descarte de resíduos, além de mínimo preparo e manipulação da amostra. Neste estudo, a termografia no infravermelho foi, pela primeira vez, combinada com a entalpimetria visando determinação de diferentes analitos (p. ex., ferro, cálcio, sódio, cloretos e acidez) em alimentos. Desta forma, a chamada entalpimetria no infravermelho (TIE, do inglês *Thermal Infrared Enthalpimetry*) foi estabelecida empregando reações de neutralização, complexação, precipitação e oxirredução, onde foi verificada a variação da temperatura gerada nas reações para a construção de curvas de calibração. A TIE foi realizada em microplacas, empregando pipeta multicanal para a adição dos reagentes e uma câmera no infravermelho para monitoramento de temperatura gerada no interior dos poços após a adição dos reagentes. Os resultados do método proposto foram comparados com aqueles obtidos através de técnicas convencionais (volumétricas) recomendadas nos compêndios oficiais. Desta forma, a TIE foi otimizada e validada para a determinação da acidez total, fixa e volátil de vinagres, e também foi aplicada na determinação simultânea de cloreto de sódio e acidez total da salmoura de picles. Os resultados obtidos mostraram boa concordância com técnicas convencionais, efetuando as análises de maneira rápida, eficaz e com potencial para ser aplicada como análise de rotina, devido à simplicidade, rapidez e elevada frequência de análise. Desta forma, foi demonstrado que a TIE pode ser considerada uma importante ferramenta para a determinação de diversos analitos em alimentos. Ainda neste trabalho, foi feita também a avaliação da TIE usando diferentes câmeras (com detector refrigerado ou não), a fim de verificar e comparar o desempenho de câmeras no infravermelho para aplicação a TIE. Verificou-se que uma câmera refrigerada apresenta melhores resultados, entretanto equipamentos sem detector refrigerado, de menor resolução e de baixo custo apresentaram resultados excelentes e promissores.

Palavras chave: termografia, entalpimetria, microplacas, infravermelho, análise simultânea.

ABSTRACT

DEVELOPMENT OF ANALYTICAL METHODS USING THERMAL INFRARED ENTHALPIMETRY

AUTHOR: Bruna Tischer
ADVISER: Juliano Smanioto Barin

Quality control of food and medicines is largely done using methods recommended in official compendia, including titration and gravimetry, which involve several negative aspects and are at odds with the principles of green analytical chemistry. Enthalpy methods are based on the enthalpy determination of chemical reactions. In the entalpimétrica analysis the quantitative determination of a reagent or catalyst is made through the enthalpy variation of a chemical reaction, that can be measured directly or indirectly. The main approaches for the entalpimetric analysis are thermometric titration, direct injection enthalpymetry (DIE) and flow entalpimetry. However, despite the availability of commercial instruments for this purpose today, these techniques have been replaced and almost disappeared from the literature, probably due to the high cost of microcalorimetry devices and the limitation of the low frequency of analysis, leading to the choice of Other analytical methods. Currently, there is a need for the development of analytical methods that provide quick results, low cost, low reagent consumption and waste disposal, and minimal sample preparation and manipulation. In this study, infrared thermography was, for the first time, combined with enthalpymetry for the determination of different analytes (eg, iron, calcium, sodium, chlorides and acidity) in foods. In this way, the so-called Thermal Infrared Enthalpyimetry (TIE) was established using neutralization, complexation, precipitation and oxidation reactions, in which the temperature variation generated in the reactions for the construction of calibration curves was verified. The TIE was performed on microplates using a multichannel pipette for the addition of the reagents and an infrared camera for monitoring the temperature generated inside the wells after the addition of the reagents. The results of the proposed method were compared with those obtained through conventional (volumetric) techniques recommended in official compendia. In this way, TIE was optimized and validated for the determination of total, fixed and volatile acidity of vinegars, and was also applied in the simultaneous determination of sodium chloride and total acidity of pickle brine. The obtained results showed good agreement with conventional techniques, performing the analyzes in a fast, efficient and with potential to be applied as routine analysis, due to the simplicity, speed and high frequency of analysis. Thus, it has been demonstrated that TIE can be considered an important tool for the determination of several analytes in foods. Also in this work, it was also done the evaluation of the TIE using different cameras (with refrigerated detector or not), in order to verify and compare the performance of infrared cameras for application to TIE. It was verified that a refrigerated camera presents better results, however equipment without refrigerated detector, of lower resolution and of low cost presented excellent and promising results.

Keywords: thermography, entalpimetry, microplates, infrared, simultaneous analysis.

SUMÁRIO

1. INTRODUÇÃO

Na análise para controle de qualidade de alimentos, a maioria das análises ainda é feita usando os métodos convencionais, os quais são recomendados nos compêndios oficiais. Dentre esses métodos, estão a titulação e a gravimetria que, apesar de sua simplicidade e largo uso, apresentam vários inconvenientes, como a necessidade de várias etapas de preparo de amostra, tempos de análise geralmente longos e exigem elevadas quantidades de reagentes. Além disso, nas titulações, muitas vezes a determinação do ponto final da reação é feito pelo analista, e erros podem principalmente se a concentração do titulado é baixa (MASINO et al, 2005).

Em decorrência desses inconvenientes, tem aumentado o interesse em métodos alternativos de análise, que estejam de acordo com os princípios envolvidos na química verde, como os que evitem ou minimizem a manipulação de elevada quantidade de amostra, que façam medições *in situ*, que abranjam a integração de processos e operações analíticas com economia de energia e redução de reagentes. Além disso, são desejados métodos que envolvam a miniaturização e automatização de processos (GAŁUSZKA et al, 2013).

A temperatura é um parâmetro importante em diversas reações químicas e processos produtivos na área de alimentos, sendo constantemente monitorada para fins de controle e garantia da qualidade tanto dos meios de produção quanto do produto final. Este monitoramento geralmente é feito usando termômetros, termopares e termistores, ou seja, métodos invasivos e que possibilitam apenas a verificação de temperatura média, de maneira pontual. A termografia no infravermelho é uma técnica de determinação da temperatura superficial de objetos que opera de forma não destrutiva, simultânea e rápida, permitindo um mapeamento de temperatura (GOWEN *et al.*, 2010; LOSKYLL *et al.*, 2012). Apesar de sua ampla aplicação em diversas áreas, tais como em medicina, microbiologia, agricultura, engenharia, materiais, entre outras, a mesma tem sido pouco explorada para análises químicas, havendo apenas uma aplicação para análises químicas através do efeito fototérmico (SUSZYŃSKI e ZARZYCKI, 2015).

O calor liberado pelas reações químicas pode ser utilizado como ferramenta analítica e, dentre os métodos que usam tal propriedade, encontra-se a entalpimetria, que consiste na determinação da variação da entalpia de uma reação química. Esta medida da variação de entalpia geralmente é feita de maneira convencional (p. ex., com termistores), usando equipamentos de alto custo como os microcalorímetros, que exigem grande quantidade de reagentes e demandam um elevado tempo de análise (STAHL, 1994).

Nesse trabalho um novo método analítico foi desenvolvido, envolvendo a combinação da análise entalpimétrica com a termografia no infravermelho. Para tanto, materiais simples e de baixo custo foram utilizados para fazer as reações, tais como microplacas descartáveis como reatores, pipeta multicanal para a adição dos reagentes e uma câmera no infravermelho para monitoramento de temperatura gerada na reação. Dessa forma, a entalpimetria no infravermelho (TIE, do inglês *Thermal Infrared Enthalpimetry*) foi utilizada como uma alternativa frente aos métodos volumétricos convencionalmente empregados para o controle da qualidade de alimentos. Várias reações foram avaliadas e diferentes aplicações foram desenvolvidas, de forma a mostrar o potencial da técnica proposta. Ainda neste trabalho diferentes câmeras no infravermelho (de diferentes resoluções e com detectores providos ou não de refrigeração) foram avaliadas para aplicação na TIE.

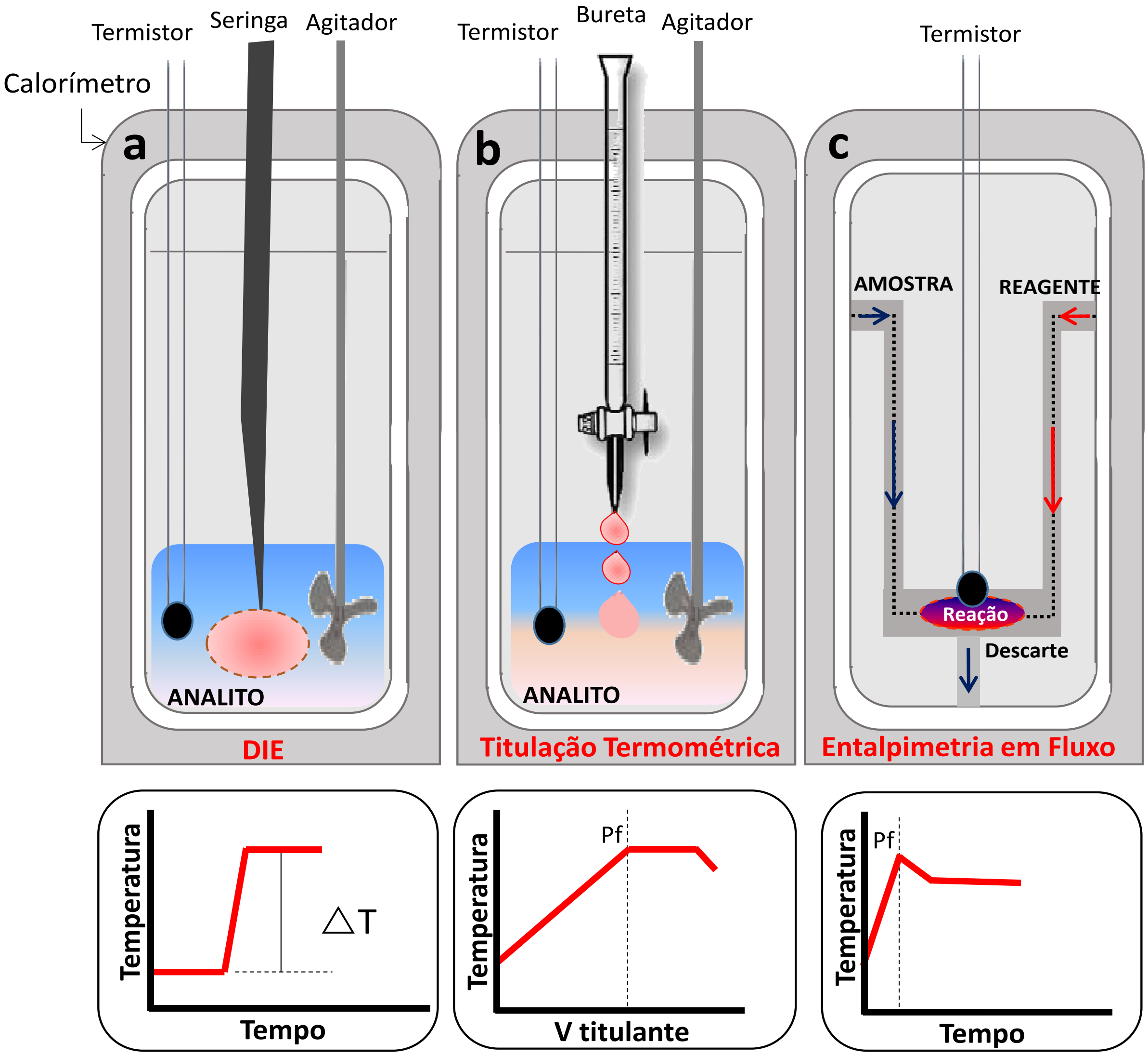
2. REVISÃO DA LITERATURA

2.1 ENTALPIMETRIA

De acordo com a IUPAC (União Internacional de Química Pura e Aplicada, do inglês *International Union of Pure and Applied Chemistry*), análise entalpimétrica é a designação genérica para uma família de métodos de análise, na qual a variação de entalpia de uma reação química é medida direta ou indiretamente, a fim de realizar uma determinação quantitativa de um reagente ou catalisador. Nessa técnica, normalmente, ao menos um reagente encontra-se em solução ou na forma líquida. As principais técnicas de análise entalpimétrica são a titulação termométrica, a entalpimetria por injeção direta (DIE, do inglês Direct Injection Enthalpimetry) e a entalpimetria por injeção em fluxo (GRIME, 1981), conforme mostrado na Figura 1.

Na titulação termométrica, o titulante é adicionado em um recipiente (por exemplo, calorímetro adiabático), resultando em uma mudança da temperatura da solução, a qual é usada para localizar o ponto final da reação. Na DIE, a reação ocorre através da injeção de uma solução contendo um excesso estequiométrico de reagentes para o interior de um recipiente contendo a solução da amostra. A diferença de temperatura é determinada antes e após a injeção, a qual está diretamente relacionada com a quantidade do reagente limitante, geralmente o analito (BOUDEVILLE e BURGOT, 1992; NAJIB et al., 2007). Na entalpimetria por injeção em fluxo, o contato entre a solução em excesso estequiométrico e a solução da amostra é realizado no interior da tubulação de tais sistemas, empregando, normalmente, um termístor para determinação da variação de temperatura.

FIGURA 1: Descrição simplificada do arranjo experimental e sinal analítico obtido pelas principais técnicas de análise entalpimétrica: a) entalpimetria por injeção direta; b) titulação termométrica e c) entalpimetria por injeção em fluxo.



O termístor pertence à classe de componentes eletrônicos semicondutores, cuja resistência elétrica é fortemente influenciada pela variação da temperatura (Morris e Langari, 2016). O termístor é um sensor de grande sensibilidade, boa repetitividade e exatidão, mas é sensível a fatores físicos e químicos que podem interferir no seu desempenho e na sua curva de resposta, visto que a medida é feita por contato com a amostra. Condições operacionais adversas, tais como excesso de temperatura, danos mecânicos ou corrosão podem causar alterações irreversíveis na correlação resistência versus temperatura do dispositivo (VARIĆAK e SAFTIĆ, 1960). A maioria dos termistores disponíveis comercialmente limita uma operação na faixa entre -60 °C e 300 °C, podendo apresentar danos em temperaturas superiores. Além disso, os mesmos podem apresentar um grande tempo de resposta, o que depende das dimensões, das propriedades físicas dos materiais constituintes e, também, das propriedades físicas e dinâmicas do meio em que o termistor está inserido. Devido a estas limitações, torna-se importante que este dispositivo seja calibrado freqüentemente, visto que há muitos fatores que podem levar a um erro na medição (MACKLEN, 1991; IBRAHIM, 2002). Em decorrência destes inconvenientes, torna-se favorável o uso de novos métodos ou dispositivos para o monitoramento da temperatura nas reações entalpimétricas que evitem o contato com as soluções.

Dentre as técnicas entalpimétricas clássicas, as titulações termométricas dominaram as aplicações até 1964, quando a DIE foi introduzida, onde vários desenvolvimentos foram realizados com o objetivo de encontrar uma instrumentação adequada para esta finalidade. Em relação às demais técnicas entalpimétricas, a DIE apresenta algumas vantagens devido à simplicidade, rapidez e a possibilidade de executar a análise em apenas uma etapa. Entretanto, este método utiliza maiores quantidades de reagentes devido à necessidade de adição da solução em excesso estequiométrico. Equipamentos disponíveis para esta finalidade estão disponíveis ainda hoje, porém as técnicas entalpimétricas foram substituídas e quase desapareceram da literatura, devido ao custo elevado de dispositivos de microcalorimetria e pela baixa frequência de análise, tendo seu uso limitado, levando a escolha de outros métodos analíticos (ADÃO *et al.*, 2012; ZHANG *et al.*, 2016).

Devido a todos estes inconvenientes, fica evidente a necessidade de novas formas de efetuar as análises entalpimétricas, de forma a simplificar o método e torná-lo aplicável a uma maior quantidade de amostras e reações. Desta forma, o método proposto neste estudo introduz a termografia no infravermelho aliada ao uso de microplacas para as reações de forma a monitorar a temperatura de maneira simples, rápida e simultânea. Sendo assim, torna-se importante conhecer os principais aspectos da termografia no infravermelho para ampliar as possibilidades de aplicação nas análises entalpimétricas.

2.2 TERMOGRAFIA NO INFRAVERMELHO

A termografia no infravermelho é uma técnica de monitoramento da temperatura superficial dos objetos, sendo efetuada através da medida da radiação infravermelha emitida pelos mesmos. Esta técnica é bem difundida como uma tecnologia de detecção, monitoramento e diagnóstico em diversas aplicações, como por exemplo, nas áreas médicas, militares, segurança e engenharia civil. No entanto, a termografia tem encontrado aplicações em outras áreas, incluindo a área de alimentos (LAMPRECHT *et al.*, 2002). Tradicionalmente, o monitoramento de temperatura dentro da indústria de alimentos para diversos processos (p. ex., cozimento, pasteurização, esterilização) utiliza métodos invasivos, como termopares, termômetros e termistores, que fornecem informações limitadas, ou seja, medidas pontuais de temperatura. Já para a termografia, as determinações de temperatura podem ser realizadas sem contato, permitindo a avaliação da distribuição da temperatura em uma determinada superfície, em uma espécie de mapeamento, o que a torna cada vez mais atrativa(NOTT e HALL, 1999). Além disso, a velocidade de aquisição de imagens é elevada, podendo chegar à kHz, o que a torna esta técnica especialmente adequada para explorar mudanças rápidas de temperatura (CUIBUS *et al.*, 2014). Na literatura estão disponíveis alguns estudos e aplicações da termografia em alimentos, como por exemplo, para o controle da temperatura de frangos durante o aquecimento; na detecção de infestações por *Cryptolestes ferrugineus* dentro de grãos de trigo; no controle de qualidade de maçãs e no monitoramento de defeitos de tomate; controle de defeitos na pigmentação de carnes e, também, na determinação do tempo de secagem de frutas cítricas (FOSTER *et al.*, 2006; WANG *et al.*, 2006; CUIBUS *et al.*, 2014). Apesar dos resultados promissores obtidos, pode-se dizer que o uso da termografia no infravermelho na área de alimentos ainda é incipiente.

Como é sabido, todos os objetos emitem radiação infravermelha e a intensidade desta depende da temperatura do objeto e da capacidade de emitir radiação. Esta capacidade pode ser chamada de emissividade e é definida como a razão entre a energia emitida a partir de um objeto para aquela de um corpo negro na mesma temperatura, podendo variar de 0 (corpo branco perfeito) a 1 (corpo negro perfeito). Todos os materiais com uma temperatura acima do zero absoluto (-273,15 ºC) emitem radiação infravermelha, sendo que quanto maior for a temperatura do objeto, maior será a quantidade de radiação emitida pelo mesmo (HOLST, 2008). A radiação infravermelha faz parte do espectro eletromagnético e compreende a região do próximo (0,75-2,5 µm), médio (2,5-8 µm), distante (8-15 µm) e extremo (15-100 µm). Sistemas de TI baseados nos comprimentos de onda entre 8 e 15 µm, apresentam sensibilidade máxima para temperaturas de objetos situadas próxima à ambiente (NG, 2009).

Há dois tipos principais de termografia, a termografia ativa e a passiva (GOWEN *et al.*, 2010). A termografia passiva é caracterizada pela simples utilização da radiação emitida pelo objeto para monitoramento da temperatura. A na termografia ativa, a temperatura é determinada após a aplicação de um estímulo sobre uma superfície, que pode ser realizado através de uma fonte de calor, radiação eletromagnética, ultrassom, entre outros. Em todos os casos, o objetivo é a produção de um diferencial térmico no corpo, permitindo visualizar regiões com características diferentes, como por exemplo, defeitos em materiais (WIECEK, 2005).

Os detectores de radiação infravermelha mais comuns para a termografia podem ser divididos em dois grupos, os não resfriados (microbolômetros) e os resfriados para câmeras de alta sensibilidade (detectores térmicos e quânticos) (DONG *et al.*, 2003; TOPALOGLU *et al.*, 2013). O detector térmico absorve o fluxo de radiação incidente e a energia dissipada aumenta a temperatura do detector para alterar algumas propriedades físicas mensuráveis (por exemplo, a sua resistência) (USAMENTIAGA *et al.*, 2014). O detector quântico consiste de um cristal semicondutor, sendo este um contador de fótons que é igualmente sensível a todos os fótons que têm a energia mínima necessária para liberar um elétron de sua estrutura cristalina (GADE e MOESLUND, 2014). Detectores resfriados normalmente empregam fluídos criogênicos, onde o resfriamento se faz necessário para o funcionamento dos materiais semicondutores utilizados. As desvantagens de câmeras de infravermelho resfriadas estão relacionadas com o elevado custo operacional e baixa portabilidade. Além disso, o resfriamento é demorado, pois a câmera pode dispender vários minutos para esfriar antes da operação. Entretanto, as mesmas proporcionam qualidade de imagem superior em comparação com aquelas não arrefecidas.

Detectores não resfriados usam sensores que trabalham por mudança de resistência, tensão ou corrente. O microbolômetro é um tipo específico de resistência usada como um detector não resfriado, geralmente construído com materiais de óxido de vanádio (VOX) ou de silício amorfo (FIÈQUE *et al.*, 2007). Mudanças na temperatura são convertidas em sinais elétricos e transformadas em uma imagem. Câmeras termográficas com detectores não resfriados (microbolômetros) são mais compactas e menos dispendiosas que aquelas de detectores resfriados. Um dos parâmetros mais importantes é o menor custo desses detectores, porém, sua resolução e qualidade de imagem tendem a ser mais baixas do que os detectores arrefecidos (KESIM *et al.*, 2014).

Na escolha de câmeras de infravermelho para medidas de temperatura, é importante levar em consideração alguns parâmetros que podem afetar no desempenho da câmera, como por exemplo, a faixa de temperatura de operação, o NETD (do inglês *noise equivalent temperature difference*), que representa a sensibilidade térmica, o IFOV (do inglês *instantaneous field of view*) ou resolução espacial (em mrad), o número de *frames* (número de quadros fornecidos por segundo, em Hz), o MDTD (do inglês *minimum detectable temperature difference*) que demonstra a habilidade de visualização de pequenos objetos com pequenas diferenças de temperatura e o tempo de integração da imagem (em s).

Apesar da importância desses parâmetros no desempenho de uma câmera termográfica, normalmente não há possibilidade de ajuste dos mesmos. Entretanto, alguns parâmetros que podem interferir na qualidade das medidas, como a emissividade, a distância entre a câmera e o objeto, o ângulo de observação, a temperatura refletida, umidade relativa e a temperatura atmosférica devem ser consideradas na padronização dos experimentos. Maiores informações sobre a influência desses parâmetros nas medidas termográficas podem ser encontradas na literatura e nos manuais de operação dos fabricantes de câmeras, assim como os métodos de otimização e padronização dos experimentos (MEOLA et al, 2017; VOLLMER e MÖLLMANN, 2013; FLIR., 2010). Também é importante verificar a ausência de objetos com diferentes temperaturas em torno do ambiente onde serão feitas as medidas e, no caso da análise entalpimétrica, um dos fatores mais importantes é a estabilização da temperatura das soluções antes da análise, comprovando que a alteração da temperatura é apenas da reação e não de alguma diferença prévia de temperatura entre as soluções.

2.3 ENTALPIMETRIA NO INFRAVERMELHO (TIE)

Como a maioria das determinações realizadas no controle da qualidade de alimentos são conduzidas utilizando métodos convencionais de análise, e em decorrência destes apresentarem várias desvantagens, torna-se interessante o desenvolvimento de novos métodos de análise, entre eles o que englobem características relacionadas à química verde (SU e CHIEN, 2010).

Sendo assim, uma alternativa é a combinação da termografia no infravermelho com a entalpimetria usando microplacas descartáveis, pipeta multicanal para a adição dos reagentes e uma câmera infravermelha para monitoramento de temperatura superficial gerada na reação. Cabe ressaltar que os primeiros trabalhos desenvolvidos da TIE fazem parte dessa tese, o que ressalta o caráter inovador do trabalho. Assim, a revisão bibliográfica fica limitada aos trabalhos desenvolvidos pelo próprio grupo de pesquisa, limitando sua abrangência.

A TIE pode ser usada em várias aplicações, entre elas no monitoramento das mesmas reações empregadas nos métodos de titulação (neutralização, precipitação, redox e complexação). Assim, a determinação da acidez de vinagre, cloreto de sódio em soro fisiológico, ferro em comprimidos de sulfato ferroso e cálcio em comprimidos de carbonato de cálcio foi realizada e os resultados foram comparados com os de técnicas convencionais recomendadas em compêndios oficiais, com excelentes resultados obtidos (BARIN *et al.*, 2015). Também foi possível efetuar a determinação da acidez total, fixa e volátil de vinagres produzidos a partir de seis matérias-primas diferentes (vinho tinto, vinho branco, arroz, maçã e balsâmico) com a TIE, e os resultados foram comparados com aqueles obtidos por técnicas convencionais recomendadas em compêndios oficiais (TISCHER *et al.*, 2017). Nesse trabalho, foi efetuada uma otimização das principais variáveis que influenciam nas reações (p. ex., volume total de cada poço da placa, proporção dos reagentes e velocidade de dispensação). Como mencionado, com a TIE é possível obter um mapeamento de temperatura, sendo possível a análise simultânea de analitos a partir de reações diferentes em poços distintos de uma microplaca. Uma nova abordagem foi proposta, assim, envolvendo a adição da amostra sobre diferentes reagentes previamente adicionados na microplaca, de forma a permitir a análise simultânea da acidez total e da concentração de sal em salmouras de vegetais em conserva (Manuscrito 1). Além disso, foi feito o estudo de comparação da TIE usando câmeras no infravermelho com diferentes características de resolução, taxa de aquisição e com refrigeração no detector do equipamento (Manuscrito 2).

Portanto, uma nova abordagem é proposta no presente trabalho e para mostrar a viabilidade da TIE na química analítica, diversas análises entalpimétricas em microplacas em combinação com câmera infravermelha para monitoramento de reações foram propostas, de forma a atender os princípios da química verde e a aplicabilidade em análises de rotina.

3. OBJETIVOS

3.1 OBJETIVO GERAL

Desenvolver métodos analíticos para controle de qualidade de alimentos por meio da análise entalpimétrica no infravermelho.

3.2 OBJETIVOS ESPECÍFICOS

- Demonstrar a viabilidade da TIE em química analítica por meio da análise entalpimétrica, explorando as reações de neutralização, precipitação, oxirredução e complexação, na determinação da acidez total de vinagre de álcool, cloretos em soro fisiológico, ferro em comprimidos de sulfato ferroso e cálcio em comprimidos de carbonato de cálcio;

- Otimizar e validar a TIE para a determinação da acidez total, fixa e volátil de vinagres (álcool, arroz, balsâmico, maçã, vinho branco e vinho tinto) e comparar com metodologias oficiais (volumetria);
- Desenvolver método rápido utilizando a TIE para determinação simultânea da acidez total e cloreto de sódio de salmoura de picles de diversos vegetais;
- Comparar câmeras no infravermelho em relação a parâmetros de resolução, taxa de aquisição de imagens e refrigeração no detector;
- Aperfeiçoar parâmetros de medida da câmera infravermelha (distância, processamento dos dados, taxa de aquisição de imagens, entre outros).

ARTIGO 1:

Infrared Thermal Imaging: A Tool for Simple, Simultaneous, and High-Throughput Enthalpimetric Analysis

Periódico: Analytical Chemistry (FI: 5.886; Estrato B1)

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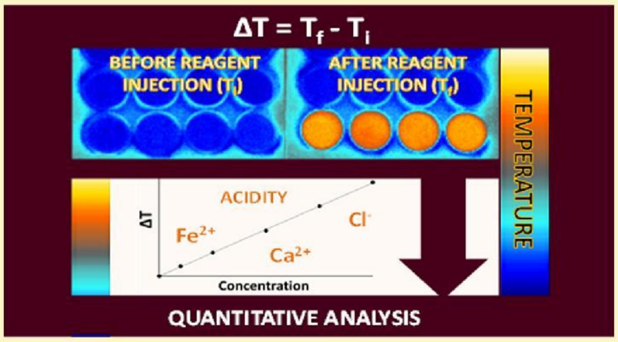
Infrared Thermal Imaging: A Tool for Simple, Simultaneous, and High-Throughput Enthalpimetric Analysis

Juliano Smanioto Barin,*[†] Bruna Tischer,[†] Alessandra Stangherlin Oliveira,[†] Roger Wagner,[†] Adilson Ben Costa,[‡] and Erico Marlon Moraes Flores[§]

[†]Departamento de Tecnologia e Ciência dos Alimentos and [§]Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria, Rio Grande do Sul, Brazil

[‡]Departamento de Biologia e Farmácia, Universidade de Santa Cruz do Sul, 96815-900 Santa Cruz do Sul, Rio Grande do Sul, Brazil



ABSTRACT: In this work, the feasibility of infrared thermal imaging (ITI) is demonstrated to show its potential application in analytical chemistry. A system of ITI was combined with disposable microplates to perform enthalpimetric analysis, which was selected as an example in order to show the reliability of this method. In this way, the novel thermal infrared enthalpimetry (TIE) method was evaluated in neutralization, precipitation, redox, and complexation reactions, with a multichannel pipet for adding the reagent and an infrared camera to monitor the temperature of multiple reactions (up to 24 simultaneous reactions) in a contactless way. Analytical signals were obtained in only 10 s, and the difference in temperature (T) before and after the reaction was used for the construction of calibration curves by use of reference solutions. More than 10 000 values were considered for the temperature determination for each reaction. The proposed method was applied for determination of the total acidity of vinegar as well as the chloride, iron, and calcium content of pharmaceuticals. The results were compared with those from conventional techniques (titration), and agreement between 96% and 101% was obtained. Sample throughput could even reach thousands of samples analyzed in 1 h. These preliminary results demonstrate the important features of TIE and possible application for other matrices and analytical parameters. The proposed TIE could be spread to cover other enthalpimetric techniques, different reactors (e.g., microfluidic and paper analytical devices), and portable devices, thus reaching other fields of chemistry.

Enthalpimetry or enthalpimetric analysis, initially defined as a subset of the field of calorimetry, is driven by the need to increase sample throughput and simplify instrumentation, thereby allowing the application of calorimetric techniques to solve routine analytical problems.[1] According to IUPAC, “enthalpimetric analysis is the generic designation for a family of analytical methods in which the enthalpy change of a chemical reaction is measured, directly or indirectly, in order to perform a quantitative determination of a reactant or catalyst”, and it is usually performed with at least one reactant in liquid or solution form.[2] This method could be performed by using three main approaches: thermometric titration, direct injection enthalpimetry (DIE), and flow injection enthalpimetry.[1−3]

In thermometric titration, a titrant is added continuously or stepwise to a solution in a vessel (e.g., an adiabatic or isoperibol calorimeter), and the enthalpy of the reaction results in the solution changing temperature, which could be used to find the titration end point.[1,2] In DIE, the reaction is initiated by injection of a solution containing a stoichiometric excess of a reagent into a vessel (e.g., an isoperibol calorimeter) containing the sample solution. The temperature is determined before and after injection, and it is directly related to the amount of the limiting reagent (usually the analyte).[1,2]

The first experiment dealing with thermometric titration was published in 1913,[4] but little attention was given to it until the use of thermistors as temperature sensors in 1953,[5] which led to several improvements to this method.[1] In this way, thermometric titrations dominated enthalpimetry until 1964, when DIE was introduced.[1,6] Between that time and 1990, several developments in instrumentation were presented in order to encourage the widespread use of enthalpimetric analysis.[7−9] However, despite the availability of commercial instruments for this purpose even nowadays,[10,11] these techniques almost disappeared from the literature at the end of the last century. It is difficult to point out the causes for this loss of interest in enthalpimetric analysis, but the high cost of microcalorimeter devices and low sample throughput limited its use for routine analysis, leading to the use of other analytical methods.[9]

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Infrared thermal imaging (ITI) is a noncontact, nondestructive technique providing temperature mapping with fast response times, which is not possible with other temperature sensors.[12] Thermal cameras do not require an illumination source, unlike other imaging systems, and they can be operated at room temperature. Images and videos can be easily recorded and processed by use of user-friendly software in order to obtain temperature values, promoting an increase in the use of ITI in various fields such as the investigation of art and historic artifacts,[13] forensic investigation,[14,15] agriculture and food industries,[12,16,17] microbiology,[18,19] medicine,[20] physics education,[21] materials characterization[22] and remote sensing.[23,24] Despite this spread in use, few applications of ITI have been found in chemistry, such as in synthesis[25] and catalysis.[26] In analytical chemistry, the application of ITI is recent and has been limited to active thermography exploring the photothermal effect in thin-layer chromatography.[27]

Therefore, a new approach is proposed in the present work to show the feasibility of ITI in analytical chemistry by means of enthalpimetric analysis. Disposable microplates were used in combination with an infrared camera for the contactless and simultaneous temperature monitoring of multiple reactions. In this way, DIE was chosen to demonstrate the potential of the proposed method in neutralization, precipitation, redox, and complexation reactions. Samples were inserted into wells, followed by addition of excess reagents by means of a multichannel pipet, and the temperature (before, during, and after injection) was recorded as an ITI video (see Supporting Information), which was used to obtain the analytical signals. Preliminary results obtained by the proposed thermal infrared enthalpimetry (TIE) method were compared with those obtained by conventional analytical techniques (titration), and the potential and challenges of this novel method are discussed.

EXPERIMENTAL SECTION

Samples, Standards, and Reagents. TIE analysis was evaluated with a variety of matrices such as vinegar and physiological saline sterile solution, as well as ferrous sulfate and calcium carbonate tablets, which were obtained from local markets and pharmacies in Brazil. Reference solutions for neutralization, precipitation, redox, and complexation reactions were prepared in water from hydrochloric acid, sodium chloride, ferrous sulfate, and calcium chloride dihydrate, respectively. All solutions were standardized before use as described in official compendia.[28,29]

Distilled and deionized water was further purified on a Milli-Q system (18.2 MΩ·cm, Millipore Corp., Bedford, MA), and this was used to prepare all standard solutions and reagents. Anhidrous calcium chloride, ceric sulfate heptahydrade, hydro-chloric acid, nitric acid, potassium biphthalate, potassium permanganate, sodium carbonate, sodium hydroxide, silver nitrate, and sodium oxalate of analytical grade were obtained from Vetec (Duque de Caxias, Brazil). Blue hydroxynaphthol, methyl orange, and phenolphthalein indicators as well as ferrous sulfate heptahydrate, sodium chloride, and sulfuric acid were purchased from Dinamica (Diadema, Brazil). Analytical-grade potassium chromate, disodium ethylenediaminetetra-acetic acid (EDTA), and tetrasodium EDTA were obtained from Sigma (Sigma−Aldrich, St. Louis, MO), and *o*-phenanthroline was from Labsynth (Diadema, Brazil).

Instrumentation. ITI was performed with a long-wave infrared camera (7.5−13.0 μm, E60 model, FLIR, Wilsonville, OR), which provided 320 × 240 pixel images with an accuracy of ±2% of reading and a maximum frame rate of 60 Hz. Images were processed by ResearchIR software (FLIR). Reactions were carried out in a commercial disposable 24-well polystyrene microplate with an internal volume of 3.4 mL for each well (Nest Scientific, Rahway, NJ). An electronic multichannel pipet (eight channels, 0.05−1.2 mL, 0.005 mL resolution; Research Pro, Eppendorf, Hamburg, Germany) was used for simulta-neous addition of reagents and a magnetic stirrer (Centauro, Atuba, Brazil) was used to homogenize solutions. The arrangement of the proposed TIE method is shown in Figure 1. In all experiments, the infrared camera was positioned 40 cm above the microplate.

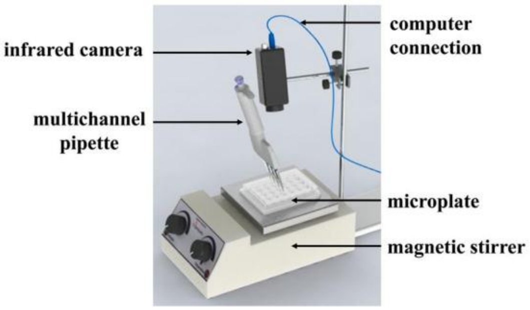

Figure 1. Experimental setup used for thermal infrared enthalpimetry (TIE).

Thermal Infrared Enthalpimetric Analysis. Reactions were performed with 1.2 mL of diluted or dissolved samples or reference solutions followed by addition of 1.2 mL of the reagent in excess. The electronic pipet was set at the maximum dispensing speed (0.5 mL·s^{-1}) and four channels were used in each dispensing step. A poly(tetrafluoroethylene)-covered magnetic stir bar (1.5 × 3.5 mm) was added into each well of the microplate to improve homogenization (500 rpm).

All reactions evaluated (neutralization, precipitation, redox, and complexation) were chosen on the basis of classic methods used for routine analysis of studied samples. For determination of the total acidity of vinegar, a calibration curve was constructed from HCl reference solutions (0.10−1.0 mol·L^{-1}, 1.2 mL), and a sodium hydroxide solution (2 mol·L^{-1}, 1.2 mL) was used in excess. Chloride was determined in a physiological saline sterile solution, with calibration via NaCl reference solutions (0.10−0.50 mol·L^{-1}, 1.2 mL) with an excess of $AgNO_3$ (1 mol·L^{-1}, 1.2 mL). The iron content of ferrous sulfate tablets was determined by use of acidified (pH = 4.0) $FeSO_4·7H_2O$ reference solutions (0.05−0.30 mol·L^{-1}, 1.2 mL) and the addition of potassium permanganate (0.2 mol·L^{-1}, 1.2 mL) that was used in excess. For calcium determination in the calcium carbonate tablets, a calibration curve was constructed with $CaCl_2·2H_2O$ reference solutions (0.10−1.0 mol·L^{-1}, 1.2 mL), and 0.6 mol·L^{-1} tetrasodium EDTA (1.2 mL) was used in excess (pH = 9.0).

For determination of total acidity in vinegar and of chloride in physiological saline sterile solution, samples were directly pipetted into wells without previous preparation or dilution. Ferrous sulfate tablets (30 units) were crushed, mixed with 0.1 mol·L^{-1} HCl, and filtered. Then the pH of the filtrate was adjusted to 4.0 with 0.1 mol·L^{-1} HCl solution and filled up to the mark with water in 200 mL volumetric flasks. The same procedure was used for the calcium carbonate tablets, but the

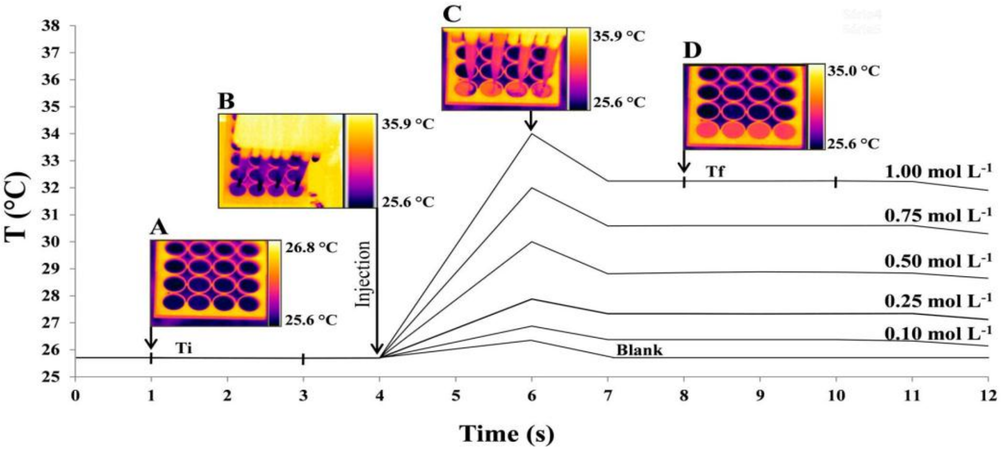

Figure 2. Enthalpogram observed for neutralization reaction. Reactions were performed with HCl reference solutions and 2 mol·L^{-1} NaOH as excess (1.2 mL for both solutions). Infrared thermal images were taken (A) before addition of reagent, (B) at initial addition of reagent, (C) during addition of reagent, and (D) after reaction.

pH was adjusted to 9.0 with 0.1 mol·L^{-1} NaOH solution with a final volume of 500 mL.

The temperature of each reaction was monitored before, during, and after the injection of the reagent in excess, and an ITI video (see Supporting Information) was used for subsequent data processing. In all measurements, the temperature change was corrected for ambient temperature (22−27 °C) and relative humidity (70−80%), with emissivity set to 0.97 as recommended by the manufacturer of the infrared camera. The temperature of the solutions before the reaction obtained from the camera data was compared with those from a mercury thermometer, and the results presented no differences. By use of the camera software, a circle with 180 pixels was used for monitoring the temperature of each well, and these temperatures were plotted to form an enthalpogram (Figure 2). The difference in temperature (T) was obtained from the equation $T = T_f - T_i$, where T_f and T_i are the final and initial temperatures, respectively. The values of T_i and T_f were determined from the mean temperature obtained 2 s before and after the reaction, respectively, as shown in Figure 2. A calibration curve (least-squares regression) was constructed from the reference solutions, resulting in the following equation for calculation of analyte concentration in the solution based on temperature measurements:

$$c_0 = (T - B_0)/B_1$$

where c_0 is the concentration of analyte in the solution (moles per liter), T is the difference in temperature of the solution (degrees Celsius), B_0 is the intercept of the calibration curve, and B_1 is the slope of the calibration curve.

Twenty-four wells of the microplate were used in all experiments. Blanks were obtained for all reagents and for the steps applied for preparation of calcium carbonate and ferrous sulfate tablets. Water was used as a blank for physiological saline sterile solution and vinegar. In all measurements, addition of the reagent in excess was performed without contact with reference and sample solutions.

Analysis by Conventional Techniques (Titration).

Determination of total acidity of vinegar was performed by titration with 0.5 mol·L^{-1} NaOH solution standardized with potassium biphthalate and phenolphthalein as the indicator.[28] The results were expressed in grams of acetic acid/100 mL of sample. Determination of chloride was performed with 0.1 mol· L^{-1} $AgNO_3$ standardized with NaCl and potassium chromate indicator.[29] The results were expressed as grams of NaCl/100 mL of sample. Determination of iron was carried out according to Brazilian Pharmacopeia,[29] by use of 20 units of ferrous sulfate tablets, which were weighed and ground. An amount equivalent to 0.5 g of ferrous sulfate powder was mixed with 20 mL of sulfuric acid, followed by the addition of 80 mL of freshly boiled and cooled water and the o-phenanthroline indicator solution. Titration was performed with 0.1 mol·L^{-1} $CeSO_4$ solution standardized with $Na_2C_2O_4$. The results were expressed as milligrams of iron/tablet. For determination of calcium in calcium carbonate tablets, around 0.1 g of the sample was weighed and diluted in 50 mL of water plus 2 mL of 0.1 mol·L^{-1} HCl, with blue hydroxynaphthol as the indicator, and the mixture was titrated with 0.05 mol·L^{-1} disodium EDTA solution standardized with $CaCO_3$ salt.[29] The results were reported as milligrams of calcium carbonate/tablet.

RESULTS AND DISCUSSION

General Aspects of Thermal Infrared Enthalpimetry.

All reactions were performed with four wells for each pipet dispensing, which were simultaneously monitored. The enthalpograms obtained are shown in Figure 2. Similar behavior by analytical signals for different reactions (neutralization, precipitation, redox, and complexation) was observed. Before injection of the reagent in excess (Figure 2A), the average temperature obtained during 2 s for each well was considered for T_i determination. Afterward, the pipet was positioned above the wells (Figure 2B), followed by injection of the reagent in stoichiometric excess (Figure 2C). In these figures, it is possible to observe that the pipet tip is in front of the camera at the beginning of the reaction and that the first aliquots of the reagent injected lead to an increase in temperature at the surface of the solution. Therefore, a homogenization time of around 1 s was necessary to remove the pipet tip and stabilize the temperature of the solution in order to allow a suitable T_f determination. Thus, T_f was determined only after this homogenization time (Figure 2D). The same time for T_i was used for the T_f determination (2 s) and it was possible to observe the homogeneity of the temperature during this time. Therefore, less than 10 s from the injection time was enough to obtain T values, and the analytical signal remained stable to measure the temperature in this interval. The time spent performing the reactions could be reduced if lower volumes of sample and reagents are used, because a reduction in pipetting time is expected. It is important to mention that blank values were negligible and that the time to obtain T was the same for all studied reactions.

For the microplates evaluated in this study, more reproducible results were obtained with the use of magnetic stirring (data not shown) due to fast homogenization of the solutions. Another important source of variability for the measurements was the multichannel pipet. As expected, the use of automatic dispensing devices allowed the same dispensing rate among TIE measurements, leading to lower deviations.

Despite the relatively low time to obtain an analytical signal in TIE (around 10 s), losses of heat to the microplate and ambient atmosphere are expected. Considering that four wells were used to dispense the reagents, the heat released from this reaction could affect the unreacted solutions positioned close to these wells. Thus, an experiment was performed with sequential addition of 2 mol·L^{-1} NaOH as the reagent in excess to a microplate filled with 0.5 mol·L^{-1} HCl (n = 24), as shown in Figure 3. As can be seen, there was no observable interference among the wells, probably due to the possibility of temperature measurement of T_i and T_f just a few seconds before and after the reaction, respectively. Therefore, the contribution of heat released from the reaction surroundings did not influence the measurements in the proposed approach.

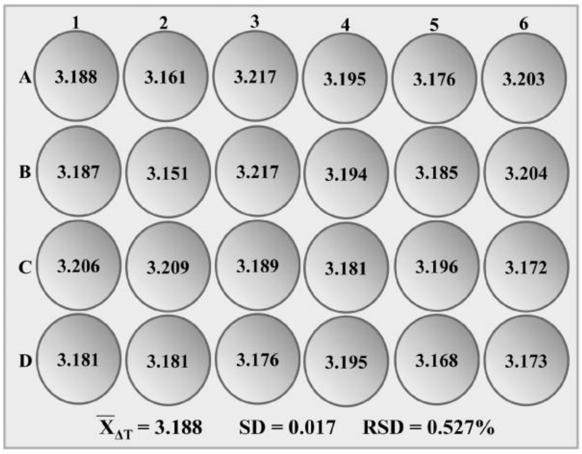

Figure 3. Temperature variation (T) of each well (values inside the circle) for 0.5 mol·L^{-1} HCl reference solution. Reactions were performed with 2 mol·L^{-1} NaOH as excess added simultaneously to four wells performing six sequential additions (n = 24; $\overline{X}$= mean; SD = standard deviation; RSD = relative standard deviation). A volume of 1.2 mL was used for both solutions.

An important finding also shown in Figure 3 is the low deviation among measurements that reached around 0.5% (n = 24). It is important to mention that, with the infrared camera, only the temperature of the solution surface was measured, and therefore the homogeneity of the solution is important in order to achieve a reliable temperature value. Considering a frame rate of 30 Hz, 180 pixels for monitoring each reaction, and 2 s for reading, around 10 800 temperature values were considered to determine the average T_i and T_f of each well. Thus, if a homogeneous solution could be obtained, the deviation expected for T is relatively low. As expected, this deviation is dependent on the number of replicates and amount of pixels used for determinating the temperature of the solution, and this could change according to the characteristics of the infrared camera.

A limitation of the proposed system is related to reaction rates. For the reactions studied (neutralization, precipitation, redox, and complexation), instantaneous and complete reactions could be achieved, leading to similar behavior and thus allowing the use of TIE for quantitative purposes. However, for lower reaction rates, the heat released from the reaction could be dissipated due to lack of isolation of the wells. Therefore, T_i and T_f could not be determined by the proposed TIE approach for lower reaction rates, and other kinds of reactors should be developed for this purpose. Fortunately, almost all reactions commonly used for titration fulfill this requirement, making TIE suitable for a wide range of applications. Therefore, widely used titration reactions such as neutralization, precipitation, redox, and complexation were evaluated in order to verify the potential of the proposed TIE method for quantitative analysis.

Calibration Curves Obtained from Different Reactions. In order to perform quantitative analysis, calibration curves were used for TIE, as shown in Figure 4. It is possible to observe good values for the coefficient of determination for all the reactions studied: neutralization, precipitation, redox, and complexation (Figure 4, panels A−D, respectively). Sensitivity was directly related to the energy released by the reaction, and therefore, higher values of enthalpy led to higher T. Another important contribution to sensitivity is the reaction rate, because lower rates could lead to losses of heat by dissipation and consequently to lower T values. When it is considered that the reaction rate could be affected by the concentration of reagents, the solutions used in excess should be evaluated in order to find their suitable concentration. As can be seen in Figure 4, the concentration of reagents in excess for all the reactions studied promoted a linear response with increasing concentration of the reference solutions, showing their suitability for quantitative analysis by proposed TIE. The limits of quantification of neutralization, precipitation, redox, and complexation reactions, determined by use of calibration curves, were 0.022, 0.038, 0.025, and 0.080 mol·L^{-1} for H^+, Cl^-, Fe^{2+}, and Ca^{2+}, respectively.

Quantitative Analysis by Thermal Infrared Enthalpimetry. Neutralization, precipitation, redox, and complexation reactions were used for determination of total acidity, chloride, iron, and calcium by both TIE and a conventional technique (titration) as shown in Table 1. The results obtained by TIE presented an agreement with conventional titration methods between 96% and 101%, showing good accuracy of the proposed method.

Deviations among the measurements obtained from TIE were generally equal to or lower than those from titrations, and this is an important finding. Considering the first study of TIE proposed here, a reduction in measurement deviations could be expected if careful evaluation of the parameters that influence them (e.g., pipetting, microplates, and infrared camera devices) is performed. In addition, the number of replicates could be easily increased in TIE, which would reduce the deviations even

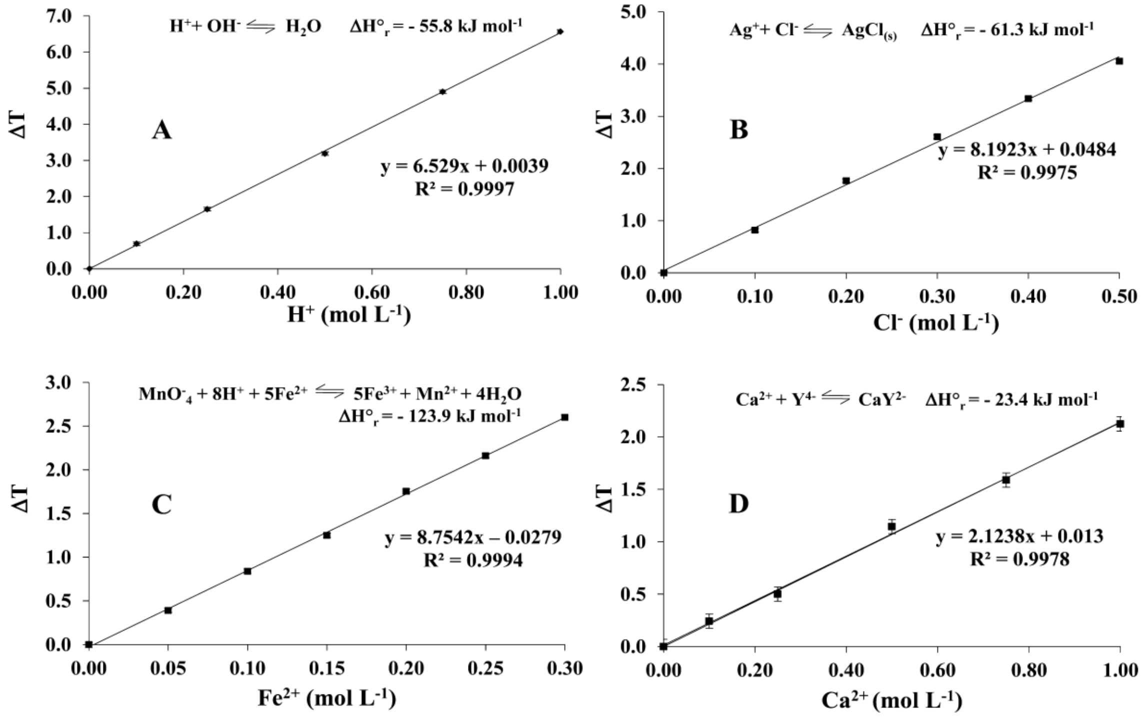

Figure 4. Calibration curves obtained for different reactions: (A) neutralization (2 mol·L^{-1} NaOH as excess), (B) precipitation (1 mol·L^{-1} $AgNO_3$ as excess), (C) redox (0.2 mol·L^{-1} $KMnO_4$ as excess), and (D) complexation (0.6 mol·L^{-1} tetrasodium EDTA as excess). A volume of 1.2 mL was used for both reference and reagent in excess solutions.

Table 1. Results for Determination of Ca^{2+}, Fe^{2+}, Cl^-, and Acidity by Proposed Thermal Infrared Enthalpimetry and Titration

sample	conventional titration method	proposed TIE method	label information
calcium carbonate tablets (mg of $CaCO_3$/tablet)	1268 ± 22	1281 ± 18	1250
ferrous sulfate tablets (mg of Fe/tablet)	52.5 ± 1.9	51.6 ± 1.8	50.0
physiological saline sterile solution (g of NaCl/100 mL)	0.890 ± 0.050	0.900 ± 0.038	0.900
vinegar (total acidity; g of acetic acid/100 mL)	4.74 ± 0.15	4.56 ± 0.09	4.20

more. However, this is not a linear correlation but it follows the square root of n.

Another important advantage of TIE in comparison to titration is the simplicity of operations with the unique step of reagent addition (injection), which is performed simultaneously for the four reactions. As mentioned before, 10 s is enough to perform TIE analysis. Considering 10 s for a complete TIE analysis of the four samples simultaneously, it is possible to analyze 24 samples per minute or 1440 per hour, which represents a much higher throughput of titration (e.g., 60 samples/h). Therefore, the sample throughput could be easily increased more than 20 times in comparison to titration. However, 12 channel pipettes and microplates with hundreds of reactors are available, and the sample throughput obtained by using TIE could be even higher.

Potential and Challenges of Thermal Infrared Enthalpimetry. Despite the preliminary and limited evaluation of TIE described in this paper, it was possible to identify important features and new possibilities for the development of this novel method. All results were obtained by manual operation of pipetting and data treatment. If these steps are automated, an increase in performance would be expected for TIE regarding the deviation of measurements and sample throughput, turning it into a powerful tool for routine analysis.

As mentioned before, the proposed method is a kind of DIE in which suitable results could be obtained, but it presents potential to be applied to other reactions (including endothermic ones) and enthalpimetric analysis techniques, such as thermometric titrations and flow injection enthalpim-etry. In this sense, other kinds of microplates or disposables devices (e.g., paper analytical devices) could be used to achieve reliable results. Insulation of the reactor devices without loss of simplicity, cost, and ease of use could be considered as ways to spread applications for analytes with lower reaction rates.

An important improvement for TIE may come from the new generation of infrared cameras, which allow high-resolution images (e.g., 1280 × 1024 pixels) and extremely high frame rates (in the kilohertz range). This equipment could improve the precision of measurements and allow for the use of enthalpimetric analysis for very fast reactions or in micro-reactors, including microfluidic devices. However, the cost of such equipment could be a limitation. On the other hand, the recent availability of low-cost (around U.S. $200) portable devices for ITI, using mobile phones as operational systems,[30,31] seems to open a way to spread the application of TIE. Therefore, the use of TIE as a process analytical technology for telemedicine and analysis in the field could be expected.

According to our findings, the most important limitations of TIE are related to selectivity of the reactions. This is very important for enthalpimetry, and its selection is a key factor for the success of this method. Although several reactions used in titrations could be carried out by use of TIE, more selective reactions such as antigen antibody and enzyme substrate could be used, allowing its use in even more complex matrices.

It is important to highlight that TIE could be used for evaluation of reactions without the interference of color, turbidity, or other components from the matrix. In this way, it is expected that for complex matrices a previous sample preparation step (e.g., filtration, dilution) could be avoided. The use of reactions in nonaqueous medium is also possible. Depending on the heat capacity of organic solvents (in general, lower than water), higher temperatures than water medium could be reached for reactions having the same enthalpy value. In addition, TIE could be used to explore not only chemical reactions but also other chemical or physical phenomena involving heat, such as adsorption, dissolution, and phase transition, demonstrating potential for several applications.

CONCLUSIONS

The combination of ITI, microplates, and multichannel pipettes was proposed to perform simultaneous enthalpimetric analysis in a simple way, and quantitative results were obtained with good agreement with conventional techniques for neutralization, precipitation, redox, and complexation reactions. Analytical signals could be obtained from multiple reactions in only 10 s, leading to a sample throughput of thousands per hour. Despite the preliminary nature of the proposed work, it was possible to revisit enthalpimetry in a new fashion, which could lead it to spread outside the applications evaluated.

ASSOCIATED CONTENT

* Supporting Information

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AUTHOR INFORMATION

Corresponding Author

*E-mail juliano@ufsm.br; fax +55 55 3220-9445.

Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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5. ARTIGO 2:

Rapid microplate, green method for high-throughput evaluation of vinegar acidity using thermal infrared enthalpimetry

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Analytical Methods

Rapid microplate, green method for high-throughput evaluation of vinegar acidity using thermal infrared enthalpimetry

Bruna Tischer [a], Alessandra Stangherlin Oliveira [a], Daniele de Freitas Ferreira [a],
Cristiano Ragagnin Menezes [a], Fábio Andrei Duarte [b], Roger Wagner [a], Juliano Smanioto Barin [a,⇑]

▪Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil
▪Departamento de Química, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil

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abstract

Infrared thermal imaging was combined with disposable microplates to perform enthalpimetric analysis using an infrared camera to monitor temperature without contact. The proposed thermal infrared enthalpimetry (TIE) method was used to determine the total, fixed and volatile acidities of vinegars. Sample preparation and analysis were performed in the same vessel, avoiding excessive sample handling and reducing energy expenditure by more than ten times. The results agreed with those of the conventional method for different kinds of vinegars, with values of 1.7%, and 2.3% for repeatability and intermediate precision, respectively. A linear calibration curve was obtained from 0.040 to 1.30 mol L^{1}. The proposed method provided rapid results (within 10 s) for four samples simultaneously, a sample throughput of up to 480 samples per hour. In addition, the method complies with at least eight of twelve recommendations for green analytical chemistry, making TIE a promising tool for routine vinegar analysis.

1. Introduction

Vinegar is produced by fermentation, a process which yields organic acids, primarily acetic acid (Moros, Iñón, Garrigues, & de la Guardia, 2008; Ozturk et al., 2015; Thomas & Jamin, 2009). Vinegar may be classified according to the raw material used for its fermentation, whether from wine (red or white), cereals, veg-etables, fruits, honey or hydroalcoholic mixtures (Brasil., 2012; Dong, Zheng, Jiao, Lang, & Zhao, 2016). Considering vinegar's high acidity, it is unsurprising that acidity has been used as a standard parameter to assure the quality of the product (Brasil., 1985; Guerreiro, Oliveira, Ferreira, & Catharino, 2014). In general, three types of acidity are considered to measure its quality: total, fixed and volatile. Volatile acidity represents the organic acids found in vinegars that are more easily vaporized than the non-volatile (fixed) acids. Volatile acidity is often obtained by calculating the difference between measured total and fixed acidities (Ubeda et al., 2016). The Brazilian official compendium for food quality control has established that the total acid content of vinegar should be between 4.0 and 6.0% (m/v), with a minimum of 4.0% of volatile acidity, expressed as acetic acid (Brasil, 2012).

Conventional protocols recommended for and used to deter-mine the acidity of vinegars require a long time and high consump-tion of energy to perform evaporation and concentration before titration. These time-consuming methods require the attention of an analyst throughout the process, leading to low sample throughput and affecting their suitability for routine applications (Masino, Chinnici, Franchini, Ulrici, & Antonelli, 2005; Su & Chien, 2010; Zeng, Cao, Liu, Chen, & Ren, 2015). Therefore, alternative methods have been proposed in the literature to determine the acidity of vinegars, namely spectrophotometry (Kurauchi, Ogata, Egashira, & Ohga, 1996), capillary electrophoresis and ion-exchange chromatography (Castro et al., 2002), gas chro-matography mass spectrometry (Marrufo-Curtido et al., 2012) and infrared spectroscopy (Moros et al., 2008). These alternative techniques generally fulfil some of the requirements of green chemistry, as they use fewer reagents and residues that are haz-ardous to human health and/or the environment, with resulting improvements in the safety of analytical operations compared to conventional methods (de la Guardia & Armenta, 2011; Gałuszka, Migaszewski, & Namies´nik, 2013; Melchert, Reis, & Rocha, 2012). However, obtaining results requires several steps, which, com-bined with the need to use certain sophisticated instrumental methods, impairs the widespread use of such methods in routine laboratories.

⇑ Corresponding author.
E-mail address: juliano@ufsm.br (J.S. Barin).

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Recently, a new analytical technique combining infrared ther-mal imaging with enthalpimetric analysis, called thermal infrared enthalpimetry (TIE), was proposed as a method for simple, fast and high-throughput analysis (Barin et al., 2015). This technique was used in titration applications, using neutralization, redox, pre-cipitation and complexation reactions. In TIE, disposable devices (e.g., microplates) contain the reactions, while an infrared camera, without contact, simultaneously monitors the temperature of multiple reactions. Reagents were introduced by direct injection, which means that only one aliquot of reagent (e.g., sodium hydrox-ide for determining acidity) in stoichiometric excess is necessary, unlike the dropwise procedure used in titrations. Analytical signals were the differences in temperature before and after reagent addi-tion. The use of multiple dispensing devices (e.g., multichannel pipettes) allowed simultaneous reactions in several wells, leading to high throughput that possibly reaches thousands of measure-ments per hour (Barin et al., 2015).

Therefore, an easy-to-use, rapid method was proposed for determining the acidity (total, fixed and volatile) of different types of vinegars (red and white wine, alcohol, apple, balsamic and rice) by TIE. Sample preparation and analysis were performed in the same vessel (microplates), avoiding excessive sample handling and improving throughput. Samples were inserted in wells fol-lowed by addition of excess reagents by means of multichannel pipettes, and the wells' temperatures (before, during and after injections) were recorded as an infrared thermal imaging video, through which the analytical signals were obtained. The dispens-ing speed, total well volume and the ratios of reagents were evaluated in order to obtain the lowest deviation between measurements. The results obtained by TIE were compared with those obtained by conventional analytical titration methods. The parameters of merit (including green ones) were presented.

2. Experimental

2.1. Samples, standards and reagents

Samples of alcohol, apple, balsamic, rice and wine (red and white) vinegars from different manufacturers were obtained from the local market of Santa Maria (Brazil). Acetic acid was used as a reference solution to construct the calibration curve (Vetec, Duque de Caxias, Brazil). Sodium hydroxide (Dinâmica, Diadema, Brazil) was used to determine the acidity. Standardization of acetic acid was performed using sodium hydroxide previously standardized with potassium biphthalate (Vetec, Duque de Caxias, Brazil; (Morita & Assumpção, 2007). Deionized water was further purified in a Milli-Q system (18.2 MX cm, Millipore Corp., Bedford, MA, USA), and this was used to prepare all solutions and reagents.

2.2. Instrumentation

A long wave infrared camera was used (8.5–13.0 lm, FLIR E60 model, FLIR, Wilsonville, OR, USA), which provided images of 320 240 pixels at a frame rate of 30 Hz. Images were processed using ResearchIR software (FLIR). The reactions were carried out in disposable polystyrene 24-well microplates, with each well having an internal volume of 3.0 mL (Nest Biotechnology, China). An electronic multichannel pipette (eight channels, 0.050–1.2 mL, Pro Research 1200, Eppendorf, Hamburg, Germany) was used to simultaneously inject reagents, and a magnetic stirrer (Centauro, Atuba, Brazil) was used to homogenize solutions. For evaporation of the samples in order to determine fixed acidity, a water bath (Solab, Brazil) was used. Energy consumption was measured by a power meter (Fluke 43B model, Fluke Corporation, Everett, WA, USA).

2.3. Analysis by conventional methods

Total acidity was determined by titration with 0.1 mol L^{1} sodium hydroxide solution and phenolphthalein as indicator, using 10 mL of sample and 50 mL of distilled water (Brasil, 1985). To determine fixed acidity, 10 mL of each sample were added in porcelain capsules, followed by heating in a boiling water bath. The evaporation procedure was performed three times, each sample reaching a total volume of 30 mL. After cooling, 50 mL of water was added, and the titration was performed in the same manner as for the determination of total acidity. Volatile acidity was obtained as the difference between total and fixed acidities. Results were expressed in grams of acetic acid per 100 mL of sample. All determinations were performed in triplicate.

2.4. TIE analysis

Acetic acid (0.04 to 1.3 mol L^{1}, 1.2 mL) or sample (1.2 mL) solutions were added to the wells, with further addition of sodium hydroxide (2 mol L^{1}, 1.2 mL). A polytetrafluoroethylene-covered magnetic stir bar (1.5 3.5 mm) was added to each well to improve homogenization. Reaction temperatures were monitored before, during and after injection of NaOH. For all measurements, temperatures were corrected in software for ambient temperature and relative humidity using values obtained from a thermo-hygrometer. Using a tripod, the infrared camera was positioned 40 cm above the microplates. Using the camera software, a circle of 180 pixels was used to monitor the temperature of each well, and the average of these temperatures was plotted, forming an enthalpogram (Barin et al., 2015). The difference in temperature (DT) was obtained using the equation DT = T_f T_i, where T_f and T_i are the final and initial temperatures, respectively. These tempera-tures were calculated as the mean of temperature values obtained from 2 s before and after the reaction. Afterwards, a least-squares calibration curve was constructed using reference solutions, and the concentrations of analytes were obtained using this curve. The 24 wells of the microplate were used for each concentration of reference solutions or samples in all experiments (n = 24). In all reactions, water was used as a blank.

The fixed acidities of the vinegar samples were determined using the microplate wells as vessels for the sample preparation (evaporation/concentration) and quantification steps. Samples (up to 3 mL each time) were added to the microplate, which was heated in a boiling water bath. For apple, balsamic, red and white wine vinegars, a total of 7 mL were evaporated, while 20 mL of alcohol and rice vinegars were evaporated due to those samples' lower concentrations of acetic acid. After evaporation, the plates were cooled at room temperature and the residues were dissolved in 1.2 mL of distilled water, followed by addition of excess of NaOH in the same way as the determination of total acidity.

2.5. Optimization of reactions for TIE analysis

Before determining total acidity by TIE, a reaction optimization was performed by considering the total volume of solution in each well, the proportion of reactants (acid/base) and dispensing speed. The optimization was carried out using 0.8 mol L^{1} acetic acid and 2 mol L^{1} sodium hydroxide solutions. The total volume in each well was evaluated using a 1:1 proportion of acid and base, ranging from 1.2 to 2.4 mL. To evaluate the proportion of reactants, which ranged from 1:1to 5:1 (ratio of acetic acid to sodium hydroxide solutions), dispensing speed was 0.57 mL s^{1} under stirring. After optimizing the total volume and proportion of reagents, the dispensing speed of the multichannel pipette was evaluated from 0.2 to 0.71 mL s^{1}. Results were evaluated using the relative stan-dard deviation (RSD) of measurements. The method was validated using white wine vinegar, which was randomly selected, and the validation followed the Eurachem Guide (Eurachem., 2014), focus-ing on linearity, working range, precision (repeatability and intermediate precision), limit of detection (LOD) and limit of quan-tification (LOQ). Repeatability was evaluated with the same analyst and equipment over 12 replicates on the same day of analysis. Intermediate precision was evaluated by three analysts using the same equipment over three days, with 12 replicates each day. Accuracy was evaluated by comparing the values obtained from TIE with those obtained by the conventional method.

3. Results and discussion

3.1. Optimization of reaction for TIE analysis

An optimization of the neutralization reaction before TIE analy-sis evaluated the influence of total well volume, acid/base ratio and speed of reagent injection in stoichiometric excess. The results are shown in Fig. 1; these parameters affect the deviation of measure-ments. The lowest deviations were achieved using 2.4 mL of total volume in wells. Larger volumes could not be used due to difficul-ties in stirring and the formation of bubbles, which impaired the measurement of temperature.

An acid/base ratio of 1:1 (v/v) provided better results in terms of RSD, probably through the fast and uniform mixture of solutions. Using a larger volume of acetic acid, the temperature increased in the upper part of the solution while the bottom remained cool, requiring longer time for homogenization and leading to higher deviations. Regarding the dispensing speed of sodium hydroxide, the lowest deviations were obtained with 0.57 mL s^{1}. Since the reaction is initiated exactly when pipetting starts, faster dispensa-tion rates provided better measurements, because the pipette tips are maintained above the wells for a shorter time, improving the mixing of solutions. With longer dispensation times the tips remained on the well surfaces and mixture occurs slowly, impair-ing the measurement of temperature by the IR camera. The param-eters which provided the lowest deviation among measurements (total volume of 2.4 mL, acid/base ratio of 1:1 and dispensing speed of the sodium hydroxide solution of 0.57 mL s^{1}) were chosen for subsequent experiments.

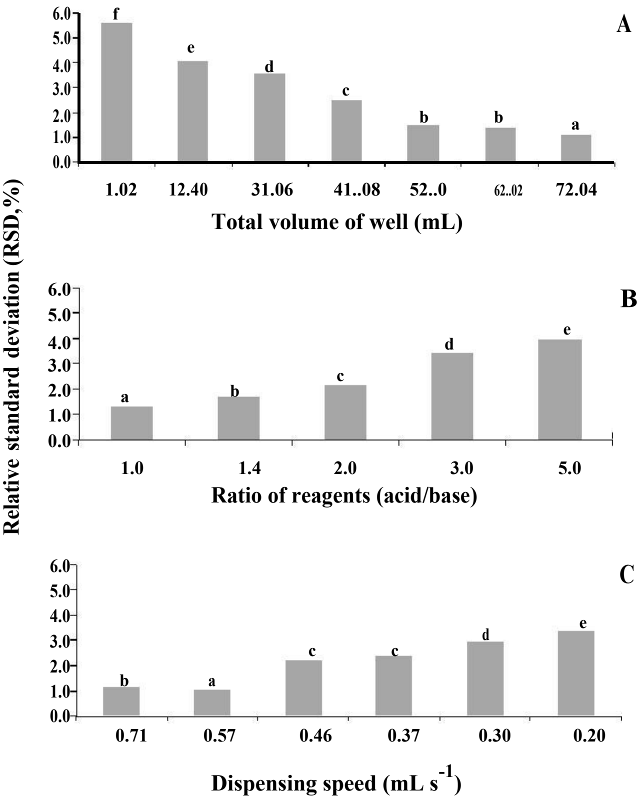

Fig. 1. Influence of total volume (A), ratio of reagents (B) and dispensing speed (C) on the relative standard deviation (RSD) of TIE measurements (n = 24). Results were obtained using 0.8 mol L^{1} acetic acid and 2 mol L^{1} sodium hydroxide solutions. Same letters indicate that data did not differ statistically from conventional methods of measurement (Tukey test, $p > 0.05$).

3.2. Determination of the acidity of vinegars

As shown in Table 1, all values obtained by TIE for the acidities of six types of vinegar were in good agreement (from 99% to 103% for total acidity, 96–102% for volatile acidity and 93–108% for fixed acidity) with conventional titration; no significant differences were observed between these methods (Student's t-test, $p > 0.05$). All vinegars presented acidity values in accordance with the require-ments of Brazilian law (Brasil, 2012).

Measurement deviations were consistently lower for TIE, prob-ably due to the integration of analytical operations (sample intro-duction, evaporation/concentration, determination) in the same vessel and the easier possibility of performing several replications. With TIE, only 3 min were enough to perform 24 replications (using all wells of a microplate) to determine total acidity, while when using titration, around 10 min are needed to perform a trip-licate. Therefore, using TIE, the number of replicates (n) may be easily increased, with a proportional reduction of deviation among measurements. It is important to mention that this reduction in deviations is not linear, following the square root of n.

Validation parameters were obtained using white wine vinegar (manufacturer B), which was chosen randomly. As can be seen in Fig. 2, the calibration curve presented good linearity ($R^2 = 0.9994$) within the range of reference solutions evaluated from 0.040 to 1.30 mol L^{1}. The LOD (3r) and LOQ (10r) were determined from the calibration curve data, obtaining values of 0.007 and 0.033 mol L^{1} of acetic acid, respectively. The precision evaluated repeatability and intermediate precision had values (RSD) of 1.7 and 2.3, respectively, which are suitable for the quality control of vinegars.

3.3. Benefits of TIE

As shown in Table 2, TIE displays marked benefits in all param-eters involved, from sample preparation to determination. The sample volume used with TIE was up to 8.3 times smaller than conventional methods, though a slightly higher amount of NaOH was consumed due to the TIE method's use of an excess (molar concentration) of this reagent. However, the use of direct injection approach for TIE avoid the need to introduce NaOH stepwise as in titration, leading to faster analysis. Using the proposed method, four samples may be analysed simultaneously in each reagent injection, reaching a sample throughput one order of magnitude higher than titration and demonstrating the usefulness of TIE for routine analysis. Therefore, the proposed method could easily be adapted to routine analysis and, if automated, could reach thou-sands of samples analysed per hour.

TIE is not affected by cloudy or strongly coloured samples (such as balsamic vinegar), because the reaction is monitored not using colour change or light absorption, but through temperature varia-tion. The contactless measurement of temperature is another important feature, because there was no need to wash or clean the device used for temperature measurement. Thus, the ease-to-use and applicability to different types of vinegars could be consid-ered important advantages of the proposed TIE method.

Table 1
Results (mean ± standard deviation) for total, fixed and volatile acidity obtained for different vinegars using TIE (n = 24) and conventional method (n = 3).

Vinegar type	Manufacturer	Total acidity[a] (%)		Fixed acidity (%)		Volatile acidity (%)	
		TIE	CONV	TIE	CONV	TIE	CONV
Alcohol	A	4.53 ± 0.07	4.56 ± 0.16	0.019 ± 0.002	0.017 ± 0.008	4.51 ± 0.07	4.55 ± 0.16
	B	4.43 ± 0.04	4.44 ± 0.11	0.016 ± 0.003	0.015 ± 0.007	4.42 ± 0.04	4.43 ± 0.11
Apple	A	4.34 ± 0.06	4.21 ± 0.15	0.077 ± 0.009	0.071 ± 0.025	4.27 ± 0.06	4.14 ± 0.16
	C	4.40 ± 0.11	4.42 ± 0.18	0.153 ± 0.025	0.164 ± 0.056	4.25 ± 0.11	4.26 ± 0.19
Balsamic	A	6.09 ± 0.12	6.03 ± 0.19	1.103 ± 0.075	1.015 ± 0.093	4.99 ± 0.14	5.02 ± 0.21
	C	6.03 ± 0.12	6.06 ± 0.19	1.660 ± 0.058	1.490 ± 0.077	4.37 ± 0.13	4.57 ± 0.20
Rice	A	4.50 ± 0.07	4.45 ± 0.16	0.049 ± 0.021	0.045 ± 0.033	4.45 ± 0.07	4.41 ± 0.17
	B	4.47 ± 0.09	4.41 ± 0.14	0.051 ± 0.011	0.046 ± 0.019	4.42 ± 0.09	4.37 ± 0.14
Wine (red)	A	4.33 ± 0.07	4.28 ± 0.17	0.216 ± 0.021	0.208 ± 0.042	4.12 ± 0.08	4.07 ± 0.18
	B	4.37 ± 0.04	4.32 ± 0.16	0.192 ± 0.005	0.179 ± 0.013	4.18 ± 0.04	4.14 ± 0.16
Wine (white)	B	4.56 ± 0.11	4.59 ± 0.18	0.149 ± 0.072	0.144 ± 0.095	4.41 ± 0.13	4.44 ± 0.21
	C	4.37 ± 0.06	4.34 ± 0.12	0.148 ± 0.032	0.137 ± 0.063	4.23 ± 0.07	4.21 ± 0.13

[a] Values informed in the label of vinegars: alcohol (4.3%), apple (4.3%), balsamic (6.0%), rice (4.3%), wine (red and white, 4.3%).

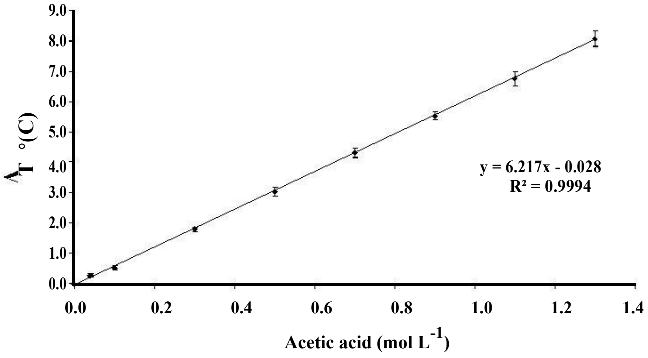

Fig. 2. Calibration curve obtained using acetic acid solutions (0.040–1.3 mol L ^{1}).

Table 2
Comparison of parameters of merit of TIE with conventional method for determination of total and fixed acidity of vinegars.

Parameters	Fixed acidity		Total acidity	
	TIE	Conventional	TIE	Conventional
Volume of sample (mL)	7	30	1.2	10
Reagent consumption (NaOH amount per sample, mg)	96	13–18	96	40–80
Time spent per run (min)	120	120	3	10
Sample throughput (samples per h)	36[a]	1.5[b]	480	18
Energy consumption (KWh)	1.49[cd]	15.87[c]	0.001[d]	–

[a] Considering three microplates in one water bath in each run.
[b] Considering three vessels in one water bath in each run.
[c] Energy consumed by water bath per sample.
[d] Energy consumed by pipette, infrared camera and magnetic stirrer.

Other important features of TIE are related to the reduced analyst supervision required during analysis, because the number of analytical steps is reduced and there is no need to clean the measuring devices. Miniaturization and the possibility of handling 24 samples simultaneously on a microplate integrates analytical operations and minimizes the risks to analysts. In addition to these advantages over titration, the energy consumption of TIE (energy expenditure per sample) is about one order of magnitude lower compared to the conventional method. Finally, the generation of a large volume of analytical waste could be avoided through the use of TIE, allowing proper disposal. Considering these features, the proposed TIE method complies with at least eight of twelve recommendations of green analytical chemistry (Gałuszka et al., 2013), making TIE a promising tool for routine applications.

4. Conclusions

The combination of infrared thermal imaging with enthalpimetric analysis could be considered a promising alternative for the determination of acidity of vinegars, especially for routine analysis. The results obtained agreed with those obtained from conventional methods, but with fewer steps and time required for analysis, leading to a sample throughput one order of magnitude higher. In addition, energy expenditures were about 10 times lower and the amount of analytical waste could be reduced, important aspects of TIE as a tool for green analytical chemistry. In this way, TIE could be reliable, easy-to-use and green alternative method for routine analysis.

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6. MANUSCRITO 1:

One-Shot Simultaneous Determination of Acidity and Salt Content of Pickled Vegetable Brine by Using Thermal Infrared Enthalpimetry

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One-Shot Simultaneous Determination of Acidity and Salt Content of Pickled Vegetable Brine by Using Thermal Infrared Enthalpimetry

[a] *Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil.*

[b] *Departamento de Biologia e Farmácia, Universidade de Santa Cruz do Sul, 96815-900, Santa Cruz do Sul, RS, Brazil.*

*Corresponding author. Fax: +55 55 3220-9445

E-mail address: juliano@ufsm.br

ABSTRACT

A rapid and high throughput method was developed for determination of total acidity and salt content of brine from pickled vegetables (containing cauliflower, cucumber, carrot and onion) using thermal infrared enthalpimetry (TIE). An infrared camera was combined with disposables microplates to perform enthalpimetric analysis using multichannel pipette for reagent addition. Microplates were charged with a stoichiometric excess of reagents (NaOH and $AgNO_3$) with subsequent addition of sample. The temperature rise of reactions was measured instantaneously and the total acidity and salt content of brine were obtained simultaneously by comparison with calibration curves. For total acidity determination, calibration curves were established with both lactic and acetic acids with no difference between them. An interference study was performed and interferences of brine composition were not observed. The obtained results by TIE analysis were compared with methods recommended in official compendia with agreements between 94 and 103%. Using the proposed approach, only 3 min were enough to perform twelve measurements of both total acidity and salt content, and therefore, 240 measurements per hour could be reached. For the same number of measurements by conventional method (titration) one or two days of work were required, showing the feasibility of method for rapid analysis.

Keywords: acidity, salt content, food, infrared thermography, infrared thermal imaging, rapid analysis

1. INTRODUCTION

Pickled vegetables are food prepared with or without bark and coated with a kind of brine, which could contain some organic acids (e.g. acetic and lactic acids) sugar and others condiments (MAPA, 1977; Medina et al., 2016). These acids could be intentionally added or formed *in situ* by fermentation and together with salt give a solution with preservative action against microorganisms and the effect of pickling, impacting sensorial and nutritional properties of product (Montaño et al., 2016). Therefore, these substances are closely linked to conservation, identity and quality of the product and are often monitored in pickled vegetables (Lopez-Moreno et al., 2016; Tôrres et al., 2011; Vincente et al., 2014)

The salt and total acidity of brine are generally determined by conventional techniques using precipitation and neutralization titrations, respectively. In spite of their simplicity, these methods are time-consuming and require constant analyst supervision leading to low throughput. In addition, the detection of the turning point is difficult for colored or cloudy samples leading to unsatisfactory results due to difficulties in titration end point determination (Tôrres et al., 2011). Potentiometry could be used to overcome these limitations, but the organic matter, particulates and other substances presented in brine could be deposited on the surface of electrodes, and an additional cleaning step should be used. It is important to highlight that these drawbacks do not impair the acidity and chloride determination by titration, but they increase the number of steps, analyst attention and time for analysis. Moreover, these methods often require excessive sample handling (e.g., weighing, dilution or filtration), which affect the throughput and the easy-to-use in process control and routine analysis.

Several methodologies have been developed to solve these problems using faster, automated and cost effective methods, as observed for total acidity determination of foods using near infrared spectroscopy (Santos et al., 2016) computer vision-based imaging (Tôrres et al., 2011), flow injection analysis with conductometric and spectrophotometric detection (Grudpan et al., 1998) and ion chromatography (Lopez-Moreno et al., 2016). Despite the advantages of these methods over titration, the fast, simple, direct and simultaneous determination of acidity and chloride directly in brine remains a challenge.

In this context, a new method was proposed requiring only one step for instantaneous determination of both total acidity and salt content of pickled vegetable brine using thermal infrared enthalpimetry (TIE). This technique was recently proposed as an alternative to titration using neutralization, redox, precipitation and complexation reactions (Barin et al., 2015). In TIE, disposable devices (e.g., microplates) were used as reactors and the temperature of each well was determined by using an infrared camera. The reaction was performed in a direct injection way, in which one aliquot of reagent in stoichiometric excess is added to the sample previously introduced in wells. The analytical signals were obtained by the difference of temperature before and after reagent injection. The use of multiple dispensing device (e.g., multichannel pipette) allowed the injection of reagent simultaneously in several wells, leading to a high throughput that could reach from hundreds to thousands of samples per hour (Barin et al., 2015; Tischer et al., 2017).

In this work, TIE was used in a different way, introducing the reagents (sodium hydroxide and silver nitrate solutions) in microplates with further addition of sample. Contrarily to other TIE methods, samples were pippeted directly on reagents, without any additional sample handling for instantaneous determination of both total acidity and chloride content. A study was performed in order to evaluate the influence of sample matrix on the determinations. In order to show the feasibility of the proposed “one-shot” method, pickled vegetables produced in Brazil were evaluated and the results were compared to titration methods.

2. MATERIAL AND METHODS

2.1 Samples, standards and reagents

Pickled vegetable samples (mixture of cauliflower, cucumber, carrot and onion) prepared with brine containing acetic acid or lactic acid from different manufacturers were purchased in a local market. Sodium chloride, acetic and lactic acids (Vetec, Brazil) were used as reference solutions for construction of calibration curves. For total acidity and salt determination, sodium hydroxide (Dinâmica, Brazil) and silver nitrate (Synth, Brazil) solutions in stoichiometric excess were used. For standardization of solutions potassium biphthalate (Vetec, Brazil) and potassium chromate (Vetec, Brazil) were used. Ultrapure water (18.2 MΩ cm) was produced by a Milli-Q Direct-Q 3-UV system (Millipore, Corp., USA) and it was used to prepare all solutions and reagents.

2.2 Instrumentation

A long wave infrared camera (8.5-13.0 µm, FLIR SC-305 model, FLIR, USA), which provided images with 320x240 pixels and frame rate of 9 Hz was used. Images were processed using ResearchIR software (FLIR). The reactions were carried out in disposable polystyrene microplates with 24 wells and internal volume of 3.0 mL. Electronic multichannel pipette (8 channels, 0.050 to 1.2 mL, Pro Research 1200, Eppendorf, Germany) was used for injection of sample and a magnetic stirrer (Centauro, Brazil) was used to homogenize solutions.

2.3 Analysis by conventional techniques

Total acidity determination was performed by titration with sodium hydroxide 0.1 mol L^{-1} and phenolphthalein solution using 10 mL of sample and 50 ml of distilled water as recommended in official compendium of food analysis (IAL, 1985). The results were expressed in grams of acetic or lactic acid per 100 mL of sample. The determination of salt was performed considering the chloride content of brine, using the mixture of 10 mL of sample and 50 mL of distilled water, followed by titration with 0.1 mol L^{-1} $AgNO_3$ and potassium chromate solution as indicator (IAL, 1985). Results were expressed as grams of sodium chloride per 100 mL of sample. All determinations were performed in triplicate. The solutions were standardized as recommended in official compendium (Morita and Assumpção, 2007).

2.4 TIE analysis

For the construction of calibration curves 1.2 mL of acetic or lactic acids (0.05 at 0.5 mol L^{-1}) or sodium chloride (0.05 at 0.6 mol L^{-1}) solutions were added to microplates with further addition of sodium hydroxide and silver nitrate solutions (1.2 ml of 2 and 1 mol L^{-1}, respectively). The conditions used in this study were based on the results obtained in previous work for the determination of the acidity of vinegars by TIE (Tischer et al., 2017). The procedure used for one-shot simultaneous determination of total acidity and chlorides by TIE is described in Fig. 1. A polytetrafluoroethylene covered magnetic stir bar (3.0 x 6.5 mm) was added in each well to improve homogenization. Reaction temperature was monitored before, during and after injection of samples. In all measurements the infrared camera was positioned 40 cm above of microplate using a tripod and the temperatures were corrected for ambient temperature and relative humidity. A circle with 180 pixels was used in software for monitoring the temperature of each well and the average of these temperatures was plotted forming an enthalpogram (Barin et al., 2015). The difference of temperature (ΔT) was obtained from equation $\Delta T = Tf - Ti$, where Tf and Ti are the final and initial temperatures, respectively. These temperatures were obtained using the mean of values obtained from 2 s before and after the reaction. Afterwards, a least squares calibration curve was constructed using reference solutions (Fig. 2). The 24 wells of microplate were used for each concentration of reference solutions in all experiments for both total acidity and salt determination (n=12 for each analyte). In all reactions, water was used as blank. For the evaluation of the results in relation to conventional method the Student t test was used ($p< 0.05$).

2.5. Evaluation of interferences on TIE measurements

For the construction of the calibration curves acetic acid and sodium chloride solutions were used. However, in the commercial pickled vegetable these substances are generally in solution with other ones, such as sugar (generally sucrose) and spices. Therefore, the interference of these substances on both total acidity and chloride determination was performed by using synthetic solutions with different combinations. For the study of interferences on total acidity determination, acetic acid solutions were prepared at 3.5% (m/V, maximum amount allowed by law for this type of product) and also acetic acid solution plus 3% sodium chloride (maximum amount of salt allowed by law) as well as 3.0% sucrose (amount commonly used in such product). The same approach was used for chloride determination. The results were evaluated by using analysis of variance (ANOVA) followed by Tukey test ($p \leq 0\ 05$) using the software Statistica 5.1 (Statsoft, 2004).

3. RESULTS AND DISCUSSION

3.1 Evaluation of parameters for TIE analysis

Calibration curves for total acidity determination were constructed using both lactic and acetic acids (Fig. 2), because these substances could be found in pickled vegetables brine. A linear behavior could be observed within the concentration range studied. Similar slopes were found for calibration curves with no differences between the confidence intervals showing the possibility of calibration with both acids for total acidity determination. Acetic acid solutions were chosen for this purpose because the majority of products presented brine prepared with this substance.

As can be seen in Fig. 3, there were no reasonable differences ($p>0.05$) among the temperature of solutions prepared with reference solutions alone or with other substances (acid, salt and sugar), showing the possibility to perform the direct determination of analytes without any treatment or dilution. These results could be considered an important finding, because the proposed TIE method was not influenced by the color of brine as well as the presence of dissolved solids or materials in suspension. The same behavior was observed for the deviation of measurements, which were not changed even at different concentration of concomitant substances. Therefore, there was not observed interferences in TIE measurements turning it suitable for total acidity and chloride determination in pickled vegetable brine.

3.2 Determination of total acidity and salt content of pickled vegetable brines

The proposed method using TIE was compared with conventional approach as shown in Table 1. Good agreement between TIE and conventional methods were observed (between 94 and 103%) with no statistical difference between results (ANOVA).This is an important finding, because by using the proposed method an integration of all analytical operations involved in total acidity and salt content determination was performed. This integration allowed the use of unique operation (sample pippeting) for determination of two analytes, saving time and increasing tremendously the applicability in routine operations. Considering that 3 min is enough for pippeting samples to one microplate with 24 wells, it was possible to reach 240 measurements per hour for both total acidity and salt content. On the other hand, for the same number of measurements by conventional method one or two days of work were required. Therefore, it was possible to demonstrate that using the proposed one-shot TIE method the sample throughput could be improved at least by one order of magnitude in a very simple way.

The ease of use is another important feature of this one-shot TIE method. Microplates could be charged with reagents in stoichiometric excess and stored till analysis with few (or none) time and effort needed to start analysis. In addition, the proposed method did not require a high degree of analyst skill and could be easily automated. Moreover, the use of microplate disposable devices avoided cleaning steps for reuse and the low sample amounts required for analysis reduced the generation of residues according to the green chemistry recommendations (de la Guardia and Armenta, 2011; Gałuszka et al., 2013)

It is important to mention that the proposed method could be used without any sample preparation (e.g., filtration, dilution, concentration), allowing a directly multi-analyte analysis. This is an important contribution in relation to other TIE methods proposed in literature (Barin et al., 2015; Tischer et al., 2017), following the green analytical chemistry principle that multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time (Gałuszka et al., 2013).

4. CONCLUSIONS

The application of TIE for determination of the total acidity and salt content of pickled vegetable brine allowed a rapid, high throughput and ease of use method suitable for rapid applications. The simultaneous analyte determination by TIE was feasible, being a promising method for quality control of this kind of samples. The sample throughput was increased tremendously in comparison to conventional titration methods, reducing the time for analysis at least by one order of magnitude.

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Table 1.Results for determination of total acidity and salt content of pickled vegetable brine (mean ± standard deviation; n = 12 and 3 for TIE and conventional methods, respectively).

Samples*	Total acidity (%)		Salt (%)	
	TIE	Conventional	TIE	Conventional
A1	0.583±0.030^a	0.591±0.032^a	2.056±0.042^a	2.018±0.039^a
A2	0.629±0.035^a	0.618±0.062^a	2.103±0.028^a	2.183±0.033^a
A3	0.686±0.038^a	0.675±0.044^a	2.424±0.054^a	2.359±0.045^a
L1	0.609±0.048^a	0.612±0.059^a	2.201±0.033^a	2.236±0.030^a
L2	0.573±0.058^a	0.590±0.035^a	2.024±0.048^a	2.086±0.048^a
L3	0.662±0.053^a	0.702±0.055^a	2.345±0.048^a	2.298±0.041^a

*letters A and L indicate pickled vegetables brine prepared with addition of acetic and lactic acids, respectively. Same letters indicate that data did not differ statistically from conventional methods of measurement (Tukey test, $p > 0.05$).

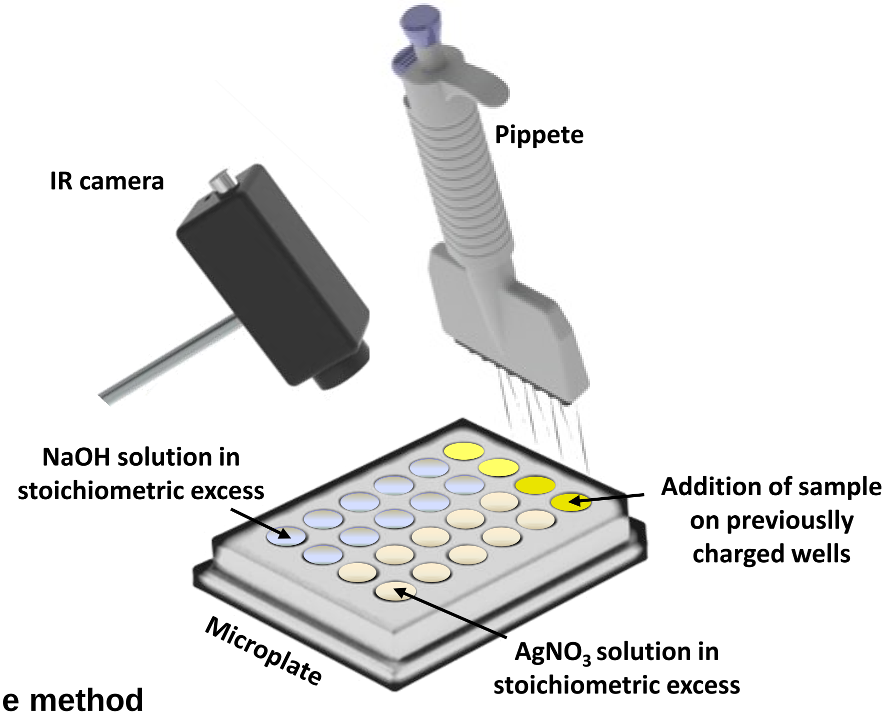
Figure captions

Figure 1. Experimental setup used for simultaneous determination of total acidity and salt content of pickled vegetable brine, using n=24.

Figure 2. Calibration curves obtained using lactic acid (A), acetic acid (B) and sodium chloride (C) solutions.

Figure 3. Influence of the composition of solution on the determination of total acidity and chloride by TIE. Reference solutions used without interfering compounds were presented as "none". Lines represented the relative standard deviation of measurements (RSD, n= 12). Same letters indicate that data did not differ statistically (Tukey test, $p > 0.05$).

Figure 1.



Stages of the method

1. Addition of 1.2 mL (per well) of excess reagent ($AgNO_3$ and NaOH) using 12 wells for each reagent;
2. Initial temperature monitoring;
3. Addition of 1.2 mL (per well) of the sample or the reagent for the construction of the calibration curve (NaCl and HCl);.
4. Monitoring the final temperature of the reaction.
5. Construction of the calibration curve and calculation of analyte concentration in the sample;

Figure 2.

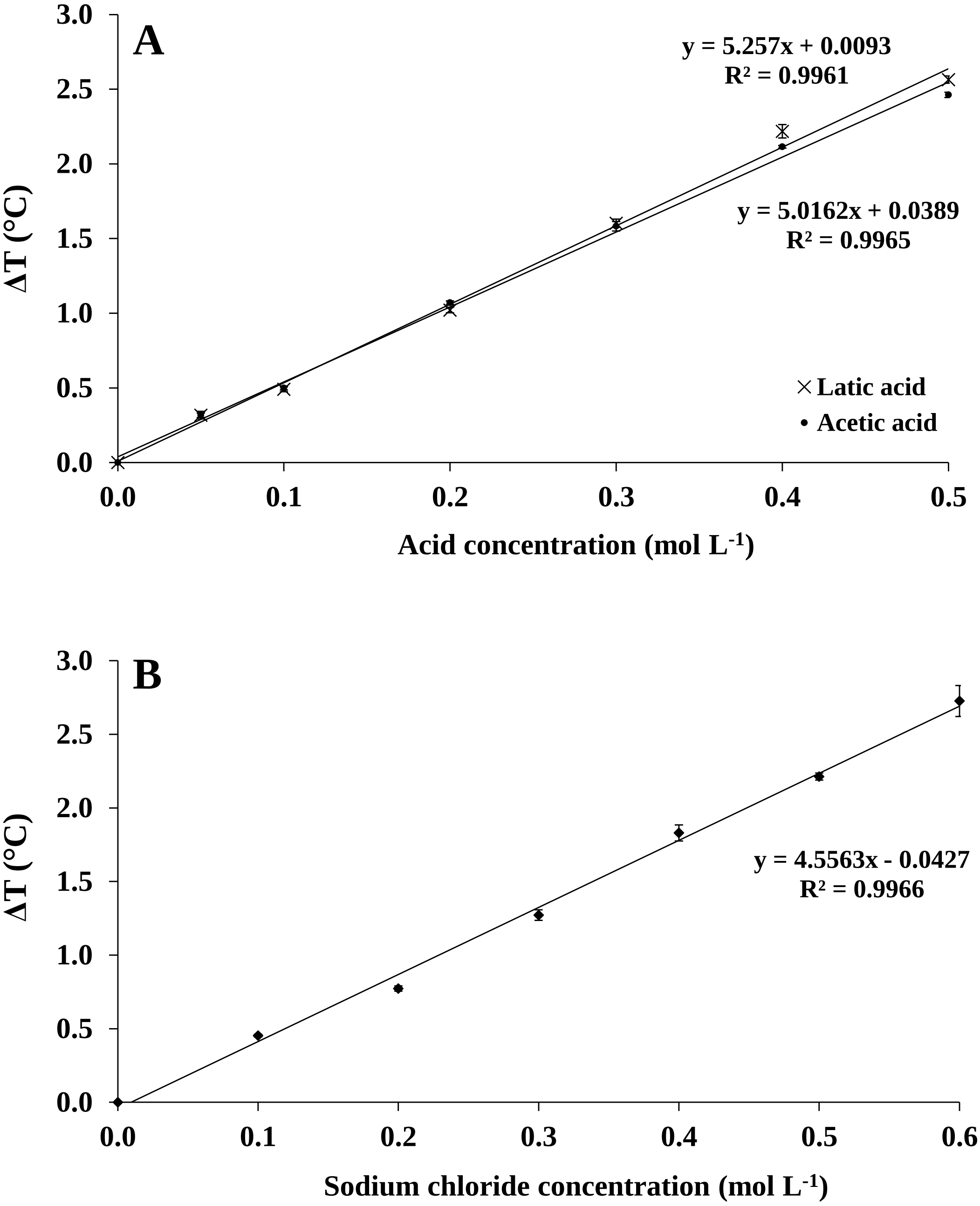
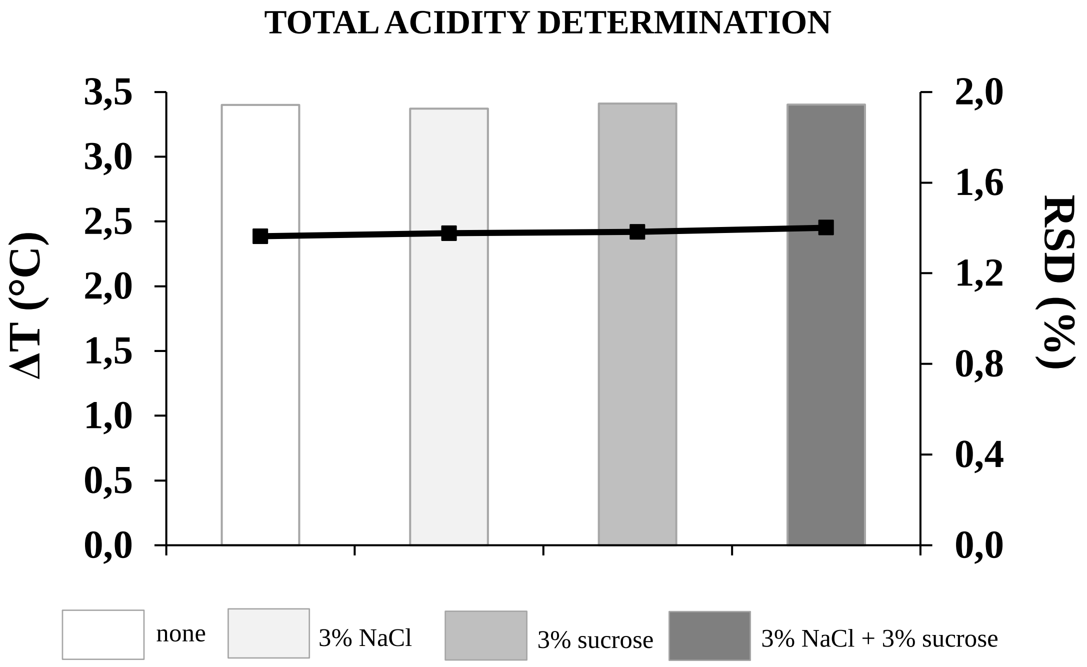
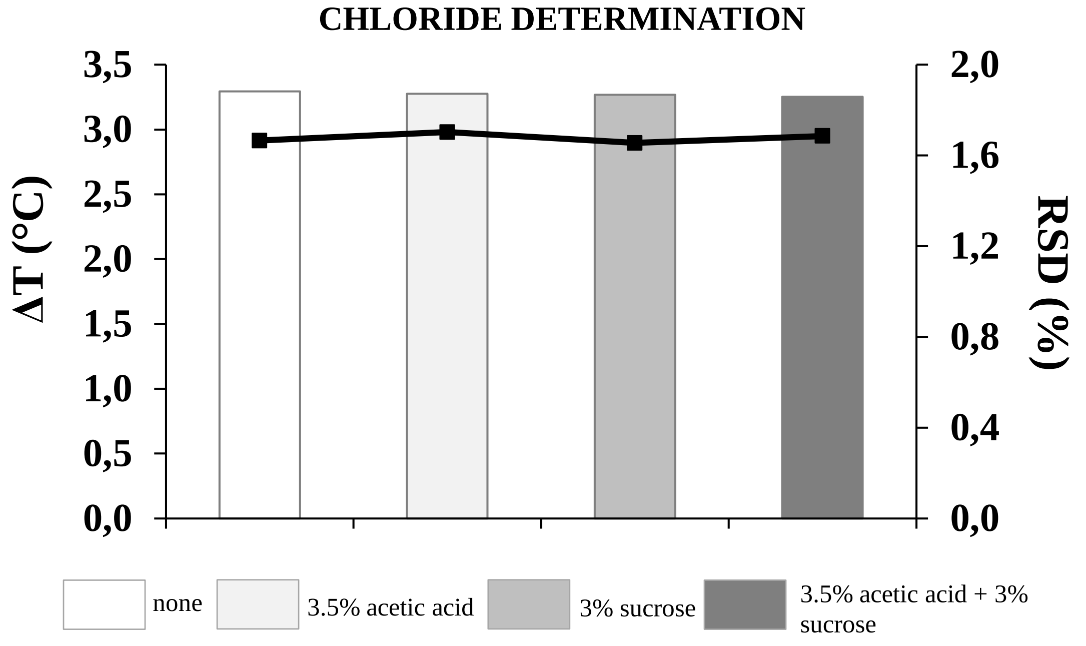

A
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ΔT (°C)
y = 5.257x + 0.0093
R² = 0.9961
y = 5.0162x + 0.0389
R² = 0.9965
× Latic acid
• Acetic acid
0.0
0.1
0.2
0.3
0.4
0.5
Acid concentration (mol L-1)
B
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ΔT (°C)
y = 4.5563x - 0.0427
R² = 0.9966
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Sodium chloride concentration (mol L-1)

Figure 3.


TOTAL ACIDITY DETERMINATION
ΔT (°C)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
RSD (%)
2,0
1,6
1,2
0,8
0,4
0,0
none
3% NaCl
3% sucrose
3% NaCl + 3% sucrose


CHLORIDE DETERMINATION
ΔT (°C)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
RSD (%)
2,0
1,6
1,2
0,8
0,4
0,0
none
3.5% acetic acid
3% sucrose
3.5% acetic acid + 3% sucrose

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7. MANUSCRITO 2:

A Critical Evaluation of Parameters for Experiments based on Thermal Infrared Enthalpimetry

Manuscrito a ser submetido para Periódico Talanta

(FI: 4.035, Estrato: A1)

A Critical Evaluation of Parameters for Experiments based on Thermal Infrared Enthalpimetry

ABSTRACT

Thermal infrared enthalpimetry (TIE), is a novel analytical technique that bring several advantages for enthalpimetric analysis as the reduction of time, energy, solvents, among others. The TIE has been developed and used in the determination of several compounds by using the determination of temperature generated in chemical reactions using an infrared camera, multi-well reactors (microplates) and multichannel electronic pipette for dispensing reagents. Uncooled thermal imaging camera had been used for TIE applications and there is no information regarding to the use of cameras with higher performance. Therefore, in this work cameras with cooled and uncooled detectors were evaluated for neutralization reactions in TIE. The kind of reactor, frame rate, number of replicates an the influence of stirring in TIE measurements were investigated. Afterwards, the determination of the total acidity of different samples (orange juice, ketchup, mustard and alcohol vinegar) was performed with both camera. The results showed that higher frame rates and larger number of pixels provided better results (smaller relative standard deviations). In relation to the different reactors, the results showed that microplates with higher size of wells lead to lower values of RSD. Stirring should be started immediately with the addition of the reagent in stoichiometric excess. The LOD and LOQ of the cameras were calculated and were adequate for the analyzed samples regarding to the total acidity, with agreement with conventional method ranging from 91 to 103%.

Keywords: enthalpimetry; cooled detector, infrared thermal imaging; infrared thermography total acidity.

1. INTRODUCTION

A novel analytical method, called as thermal infrared enthalpimetry (TIE), was recently proposed as an alternative for classical enthalpimetric methods performed in calorimeter devices. This method is based on the injection of an excess of reagent to sample solution using the temperature rise of solution caused by the heat released by the reaction with analyte. The reaction is performed in microplates and the reagent is injected simultaneously in several wells by means of a multichannel pipette. The temperature of solution of multiple wells is monitored using an thermal infrared camera and the difference of temperature before and after the injection of reagent in stoichiometric excess is used for construction of calibration curves [1, 2]. In order to assure a proper homogenization a stir bar is introduced in each well to perform magnetic stirring of solutions.

The applications of TIE were focused on the use of different reactions (e.g., neutralization, precipitation, complexation and redox ones) for determination of acidity of vinegars, chlorides, iron and calcium in pharmaceuticals and also for one-shot direct determination of acidity and salt content of pickled vegetable brines [1-3]. Other phenomena involving enthalpy changes could be used as the heat of dilution of ethanol and water, which has been used for the determination of alcoholic content of distilled beverages [4]. Despite the important features described for these applications, as the reduction of the amount of reagents, residue generation and energy consumption as well as the time for analysis, only the influence of parameters related to the volume and homogenization of solutions (e.g., total volume of solution, proportion of sample and reagent in stoichiometric excess, dispensing rate of pipette and stirring speed) on the measurements was performed.

As commented before, in TIE the measurement of temperature is performed using a thermal infrared camera. In all studies performed using TIE, only cameras with similar performance were evaluated and there is no information regarding to the use of cameras with higher performance. The most common infrared detectors can be divided in two groups, the non-cooled (microbolometers) and cooled for high sensitivity cameras (thermal and quantum detectors) [5].

The thermal detector absorbs the incident radiation flux and the dissipated energy increases the temperature of the detector to change some measurable physical properties (for example, its resistance) [6]. While the quantum detector consists of a semiconductor crystal, being it a counter of photons that is equally sensitive to all photons that have the minimum energy necessary to release an electron of its crystalline structure [7]. Cooled detectors usually employ cryogenic fluids, where the cooling is necessary for the operation of the used semiconductor materials. The disadvantages of cooled infrared cameras are related to high operating cost and low portability. In addition, cooling is time-consuming once the camera may take several minutes to cool before operation, however, it provide superior image quality compared to uncooled.

Non-cooled detectors use sensors that work by changing resistance, voltage, or the current. The microbolometer is a specific type of resistance used as an uncooled detector, usually constructed from materials of vanadium oxide (VOX) or amorphous silicon [8]. The changes in temperature are converted into electrical signals and transformed into an image. Thermographic cameras with non-cooled detectors (microbolometers) are more compact and less expensive than those with coolled detectors. One of the most important parameters is the low cost of these detectors, however, its resolution and quality of image tend to be lower than the cooled ones [9].

Several parameters could be considered for comparison of infrared cameras, because changes in the operating temperature range, thermal sensitivity, number of frames, resolution among others, could impact the quality of data obtained in infrared thermography [10-12]. Therefore, the performance of two infrared cameras equipped with cooled and uncooled detectors was evaluated regarding to TIE applications. The determination of total acidity of several food (mustard, ketchup, orange juice and alcohol vinegar) was evaluated using both cameras, and analytical parameters as accuracy, limit of detection and quantification were used for comparison of equipment. The influence of number of pixels covered in images and the image acquisition rate (number of frames) was also evaluated, as well as the number of wells and the influence of stirring before and after the addition of the solution in stoichiometric excess.

2. EXPERIMENTAL

2.1 Samples, standards and reagents

Samples of mustard, ketchup, orange juice and alcohol vinegar from different manufacturers were obtained in the local market of Santa Maria city (RS, Brazil). The mustard and ketchup samples were diluted 1:10 in distilled water previous to analysis. The samples of orange juice and alcohol vinegar were analyzed directly, without any diluition. Hydrochloric acid (37%, Vetec, Brazil) was used to prepare reference solutions for the construction of calibration curves. Sodium hydroxide (Vetec, Brazil) was used for the preparation of the solution in stoichiometric excess. The standardization of hydrochloric acid solutions was performed by using sodium hydroxide previously standardized with potassium biphthalate (Vetec, Brazil) [14]. Distilled water was used to prepare all solutions and reagents.

2.2 Instrumentation

In order to compare the results using different infrared thermal cameras, an uncooled thermal imaging camera (FLIR E60 model, FLIR, Wilsonville, OR, USA) was used. It is equipped with a uncooled microbolometer that provide images with 320 x

240 pixels at a maximum frame rate of 30 Hz. For comparison, a cooled thermal imaging camera (FLIR A6753 model, FLIR, Wilsonville, OR, USA) was used, which provided images with 640 x 512 pixels at a maximum frame rate of 60 Hz. The videos obtained were evaluated by using the ResearchIR software (FLIR). For the evaluation of the different reaction reactors, microplates with a maximum volume of 3.4, 1.6 and 0.3 mL were used, which correspond to 24 (Nest Biotech, China), 48 (Nest) and 96 (SPL Life Sciences, Korea) wells per plate, respectively. For the experiments using 24 and 48 well plates, an electronic multichannel pipette was used (8 channels, 0.050 to 1.2 mL, Pro Research 1200, Eppendorf, Germany). For the 96 wells microplate an electronic multichannel pipette (8 channels, 15 to 300 µL, Eppendorf Xplorer, Eppendorf, Germany) was used. The homogenization of solutions was performed using stir bars (3.0 x 6.5 mm) and a magnetic stirrer (Centauro, Brazil).

2.3 Determination of total acidity by conventional method

The determination of total acidity was performed by titration using sodium hydroxide solution (0.1 mol L^{-1}) and phenolphthalein as indicator, using 10 mL of sample and 50 mL of distilled water according to the official method for food analysis [15]. The results were expressed in grams of acetic acid per 100 mL of sample. All determinations were performed in triplicate.

2.4 TIE analysis

2.4.1 Evaluation of microplates with different number of replicates

In order to compare the influence of the two cameras in microplates with different number of wells, preliminary experiments were performed to achieve the optimal condition for each plate. For this purpose, the total volume in the wells and the rate of reagents addition were evaluated, using 0.5 mol L^{-1} HCl reference solution and 2 mol L^{-1} NaOH as reagent in stoichiometric excess e and independent of the number of total wells of each plate, 24 wells of each plate were evaluated for comparison. For the 96-well plate (total capacity of 0.3 mL in each well), total volumes of 0.300 , 0.250, 0.200 and 0.150 mL (with the addition of hydrochloric acid and sodium hydroxide solutions in the same ratio) were evaluated using dispensing rates of 0.96; 0.64; 0.43 and 0.32 mL s^{-1} respectively. For the 48 well plate (total capacity of 1.6 mL in each well), total volumes of 1.500, 1.200, 1.000 and 0.700 mL and dispensing rates of 0.46; 0.57 and 0.71 mL s^{-1} respectively were evaluated. For the 24 well plate (total capacity of 3.4 mL) the total volume of 2.4 mL in each well and dispensing rate of 0.57 mL s^{-1} was used as previously described [1]. For the 48 and 96 wells microplates the total volume of 1 mL and 0.25 mL, and the dispensing rates of 0.57 and 0.43 mL s^{-1} were chosen, respectively. These parameters were used in all subsequent experiments.

2.4.2 Evaluation of the area sampled from images (number of pixels)

For evaluation of the monitored area for the neutralization reaction in each well, it is possible to select in the software a circle with different size. In this way, depending on the resolution of camera and the size of wells, the number of pixels used for 24, 48 and 96 wells could be different and they can influence the results. In this way, for the 24 wells plate the number of pixels evaluated ranged from 500 to 5000 for cooled thermal imaging camera and from 10 to 450 pixels for uncooled thermal imaging camera. For 48 wells plate, the number of pixels ranged from 100 to 2000 pixels and from 10 to 200 for cooled and uncooled thermal imaging camera, respectively. For of cooled and uncooled thermal imaging camera for 96 wells microplate, from 100 to 800 and from 5 to 60 pixels were evaluated, respectively. For this determination, 0.5 mol L^{-1} HCl and 2 mol L^{-1} NaOH solutions were used.

2.4.3 Evaluation of frame rate

For evaluation of the influence of frame rate, cooled and uncooled thermal imaging cameras were used at 20, 40 and 60 and 7.5, 15 and 30 Hz, respectively . For this comparison, the 24 wells microplate was chosen, using the previously optimized conditions for this reactor. Solutions of HCl and NaOH in the concentration of 0.5 and 2 mol L^{-1} were used, respectively.

2.4.4 Influence of the number of wells selected for the measurements

The number of wells (or number of replicates, *n*) was evaluated using 24 well plate (total capacity of 3.4 mL in each well) with the addition of 1.2 mL of each solution (0.5 mol L^{-1} HCl and 2 mol L^{-1} NaOH solutions) and the cooled thermal imaging camera (640 x 512, 60 Hz). The changes in the temperature values was evaluated for 4, 8, 12, 16, 20 and 24 wells, evaluating the RSD of measurements.

2.4.5 Influence of stirring

The influence of stirring was carried out in 24 well plate with 1.2 mL of each solution (0.5 mol L^{-1} HCl and 2 mol L^{-1} NaOH solutions) and the cooled thermal imaging camera (640 x 512, 60 Hz). To check the influence of stirring on the analytical signals of temperature measurement was performed before and after the addition of the excess of reagent, with and without the use of stirring. The polytetrafluoroethylene-covered magnetic stir bar was used in each well for homogenization at 500 rpm [2].

2.4.6 Determination of total by TIE

The determination of the total acidity of the samples was performed using the 24 wells microplate, total volume of 2.4 mL in each well (1.2 mL of sample/reference and sodium hydroxide solutions) and dispensing rate of 0.57 mL s^{-1}. The calibration curve was constructed using HCl reference solutions (0.05, 0.07, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.0 mol L^{-1}) for both cameras. Afterwards, were evaluated by addition of 2.0 mol L^{-1} NaOH solutiuon. Mustard and ketchup samples were previously diluted (1:10) with water. Orange juice and vinegar of alcohol were evaluated without any pretreatment. Water was used as blank solution. The temperature monitoring was carried out before and after the addition of the reagent in stoichiometric excess, following the steps previously used for total acidity determination by TIE [2]. The final temperature was obtained 2 s after adition of NaOH solution. The temperature was corrected in the software for ambient temperature and relative humidity using values obtained from a thermohygrometer. The infrared cameras were positioned at a distance of 40 cm from the microplates.

2.5. Data evaluation

The experimental data were evaluated using analysis of variance (ANOVA) followed by the Tukey test ($p \leq 0.05$) using Statistica 7.0 software (Tulsa, USA, 2004). The limit of detection (LOD) and limit of quantification (LOQ) of curves obtained or different cameras was evaluated using followed the Eurachem Guide [16].

3. RESULTS AND DISCUSSION

3.1 Evaluation of reactor size

Considering that TIE methods were recently proposed, there are a few works, and almost of all applications were performed by using 24 wells microplate. Therefore, it is important to evaluate the impact of smaller wells on the measurements, because there is a possibility to improve even more the throughput with reduction in the amount of reagents used in TIE. As can be seen in Figure 1, the relative standard deviation

(RSD) of measurements was lower for microplate with 24 wells. Therefore, the decrease in the size of the well lead to an increase in RSD. This could be explained because the use of larger wells allowed better homogenization, but this kind of well lead to the monitoring of larger surface area and consequently a higher number of pixels for the temperature determination. In this way, lower deviations among measurements could be obtained by using 24 wells microplate.

Concerning to the type of camera used, the RSD values were lower when using cooled detector. This is expected, because the thermal sensitivity and baselines of cooled cameras presented lower values in comparison to cameras with uncooled detectors, which certainly contributes to this behavior. However, with a critical look on the results, it was possible to see that both cameras obtained RSD values lower than 2% independently of type reactor and type camera. This variation among the measurements could be considered low, showing that more simple and lower resolution camera with uncooled detector (microbolometer) could provide reliable results.

3.2 Evaluation of the area (number of pixels) for temperature monitoring

As commented before, the number of pixels used to determine the temperature could have influence on the deviations of measurements. This effect could be shown in Figure 2, and regardless the camera used (cooled or uncooled detectors), when a lower number of pixels is used higher deviation among measurements was observed. However, using the cooled thermal imaging camera it was possible to use 10 times more pixels than uncooled thermal imaging camera, because in spite of the same focal distance used for both cameras, the cooled one presented higher resolution and allowed the use of lens with lower angle leading to the use of full image and a high number of pixels.

Other important parameter for temperature monitoring is the establishment of the size of circle that cover the surface of solution. For lower size, the circles do not cover a significant area of solution's surface leading to a higher variation of mesurements. For the larger circles that covered almost 100% of the total area of well, high deviations were also observed due to the heat exchange in the borders. Then, independently of the camera and reactor size evaluated, smaller RSD values were obtained using intermediate sizes of circles for temperature monitoring, which comprise around 80% of the total amount of each well. As shown in Figure 2, the reactors with larger wells presented lower RSD, as already discussed in the study of reactor size.

3.3 Image acquisition rate (number of frames)

The number of frames or time resolution is an usual parameter that demonstrate the camera performance. The frame rate means the frequency which an unique consecutive images are produced, and it is expressed in Hz (1 s^{-1}) [14]. As showed in Table 1, higher frame rates for both cameras lead to lower RSD values. This could be explained by the number of pixels used for the determination in each temperature, which is dependent on the frame rate. For example, considering the 24 wells microplate and the cooled thermal imaging camera, the variation of the number of frames from 20 to 60 Hz changed the RSD from 1.15 to 0.78%. In this case, 2 s were considered for the temperature determination of the which lead to a total amount of 80,000 and 240,000 values of temperature for the calculation of each result. A similar behavior was observed also for uncooled thermal imaging camera, showing the importance of this parameter in TIE measurements.

3.4 Influence of number of wells analyzed

An important feature of TIE is the possibility to use a high number of replicates due to the high sample throughput, that could reach thousands of determinations per hour [2]. In this way, the papers published using TIE used a number of replicates from 12 to 24, but a systematic study to evaluate the impact of this parameter on the measurements was not performed. In this way, 24 wells microplate was used for this evaluation varying the number of wells from 4 to 24 using a neutralization reaction (Figure 3). As expected, it was observed that the increase in the number wells lead to the decrease of RSD. In general, when the replicates number is increased, the precision of the method can also increases, once the errors are minimized [17]. However, it is important to highlight that different behavior was observed for the cameras studied. The cooled thermal imaging camera allowed the use of lower number of wells without dramatic changes in RSD values, whereas for the uncooled thermal imaging camera this result is only obtained when analyzes a larger number of wells, at least eight. Therefore, when the number of replicates should be lower than eight, the use of cooled camera could provide better results.

3.5 Evaluation of the stirring effect

The influence of the stirring is shown in Figure 4. Before the addition of the reagent in stoichiometric excess, the microplate containing only hydrochloric acid solution was observed a slight increase of baseline (red line). This heating could be generated by the impact of magnetic stir bar with the internal walls of wells. Thus, for the determination of the initial temperature of solution is recommended the use of stirring only by few seconds and before the determination of temperature. As shown, for the cooled thermal imaging camera the baseline without stirring remained more constant, suffering less interference from external factors. On the other hand, the stirring was very important for temperature measurement after addition of reagent in stoichiometric excess, allowing the stabilization of final temperature in a lower time. The stirring can promote higher mass transfers, that generates faster and more efficient neutralization and the mixing involves a more intimate contacting of the solutions by means of an agitation source. [18-20].

Therefore, for temperature determination is recommended the measurement of the initial temperature before addition the excess reagent without stirring the solution, and then apply the stirring only after the addition of reagent in stoichiometric excess to assist the homogenization of solutions and enable the final temperature determination more accurately.

Both cameras showed a response with the similar profile regarding to the baseline. However, the cooled thermal imaging camera presented lower noise or variation in the initial temperature and also after the addition of the reagent in excess, but without significant difference between the cameras. This effect could be explained by the better thermal sensitivity of this camera and also by the use of a higher number of pixels for the determination of each temperature.

3.6 Determination of total acidity

The detection and quantification limits (LOD and LOQ), showed in Table 2, were calculated using the calibration curves. As already expected, the calibration curve obtained by the camera with cooled detector presented lower LOD and LOQ, probably allowed by the better signal to noise ratio. It is important to mention that the analyzed samples presented values of acidity higher than the LOD and LOQ values obtained for the two cameras. However, in some cases, when samples with very low acid values, a cooled thermal imaging camera could be useful, because it allowed the determination of lower concentrations. As shown in Table 3, the determination of acidity by TIE using both cameras did not present a significant difference ($p>0.05$) comparing to the conventional method, showing the accuracy of TIE. In addition, lower deviations were observed for some samples.

According to the Brazilian official compendium for food quality control, it is established that the total acidity content of vinegar should be between 4.0 and 6.0% (m/v) [21]. As observed in Table 3, these products presented acidity values in agreement accordance with the limits recommended by the legislation. Ketchup and mustard sauce are classified by Brazilian legislation as prepared condiments. Ketchup is defined as a sauce made with tomato paste added with spices, salt and sugar, and may contain other condiments, since mustard sauce is the creamy product obtained from mustard powder, vinegar and oil, and may contain other spices, sugar and salt. However, the legislation did not define the maximum amount of these condiments that may be present within the product. Thus, it was not possible to compare the total acidity of these products with the legislation, expressed mainly by the amount of vinegar (acetic acid) added [21-23].

4. CONCLUSIONS

The present study showed that cameras equipped with cooled detectors generally present better results for TIE regarding to precision and limits of detection and quantification. However, the results showed that simpler uncooled cameras can be used for determinations by TIE, with a negligible decrease in the performance. Both cameras provided suitable results for the determination of total acidity in food.

The reactors with higher size (microplates with 24 wells) were more suitable for application of TIE, because they provided lower deviations among measurements. Cameras with cooled detectors are recommended for reactors with lower size (microplates with 48 and 96 wells) in order to achieve best performance. The frame rate was other important parameter that affect the results, providing lower deviations were at higher rates. Other important parameter is the number of replicates; the deviations of measurements were more affected by using lower number of replicates for uncooled camera. The study of the influence of stirring was very important to elucidate the exact moment of the analysis in which the solution must be stirred in order to avoid systematic errors.

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Table I. Variation of the number of frames for different cameras and evaluation of temperature variation of the neutralization reaction.

Number of frames (Hz)	Temperature change (°C)	Relative standard deviation (RSD)
Cooled thermal imaging camera		
20	3.29±0.037	1.15
40	3.29±0.030	0.92
60	3.30±0.025	0.78
Uncooled thermal imaging camera		
7.5	3.28±0.110	1.30
15	3.31±0.092	1.09
30	3.28±0.048	0.98

Table II. Limits of detection (LOD) and quantification (LOQ) obtained from the calibration curve using 24-well plates and different infrared cameras.

Camera	**LOD**			**LOQ**		
	°C	**mol L^{-1}**	**%**	**°C**	**mol L^{-1}**	**%**
Cooled thermal imaging camera	0.09	0.03	0.10	0.31	0.06	0.21
Uncooled thermal imaging camera	0.13	0.05	0.19	0.43	0.09	0.36

Table III. Results for determination of acidity by proposed and conventional methods.

Sample	Conv. titration method (%)	TIE Cooled thermal imaging camera	TIE Uncooled thermal imaging camera
Mustard (1)	2.62±0.15	2.63±0.07	2.54±0.07
Mustard (2)	1.36±0.09	1.29±0.05	1.30±0.07
Ketchup (1)	1.88±0.08	1.85±0.04	1.73±0.04
Ketchup (2)	0.72±0.08	0.68±0.04	0.66±0.12
Orange juice (1)	1.83±0.08	1.75±0.04	1.90±0.04
Orange juice (2)	1.80±0.08	1.78±0.03	1.74±0.05
Alcohol vinegar (1)	4.13±0.12	4.08±0.03	4.05±0.04
Alcohol vinegar (2)	4.08±0.10	4.03±0.10	4.16±0.08

Figure captions

Figure 1. Evaluation of different microplates (with 24, 48 and 96 wells) and cameras for the neutralization reaction (0.5 mol L^{-1} HCl reference solution plus 2.0 mol L^{-1} NaOH; *n*=24 for each microplate).

Figure 2. Evaluation of the deviation of measurements for different microplates using different number of pixels for the different cameras.

Figure 3. Evaluation of the number of replicates (*n*) in 24 wells plate by using cooled and uncooled cameras. Neutralization reaction with 0.5 mol L^{-1} HCl reference solution plus 2.0 mol L^{-1} NaOH was used.

Figure 4. Influence of stirring before and after the addition of reagent in stoichiometric excess by using cooled and uncooled cameras. Neutralization reaction with 0.5 mol L^{-1} HCl reference solution plus 2.0 mol L^{-1} NaOH was used.

Figure 1.

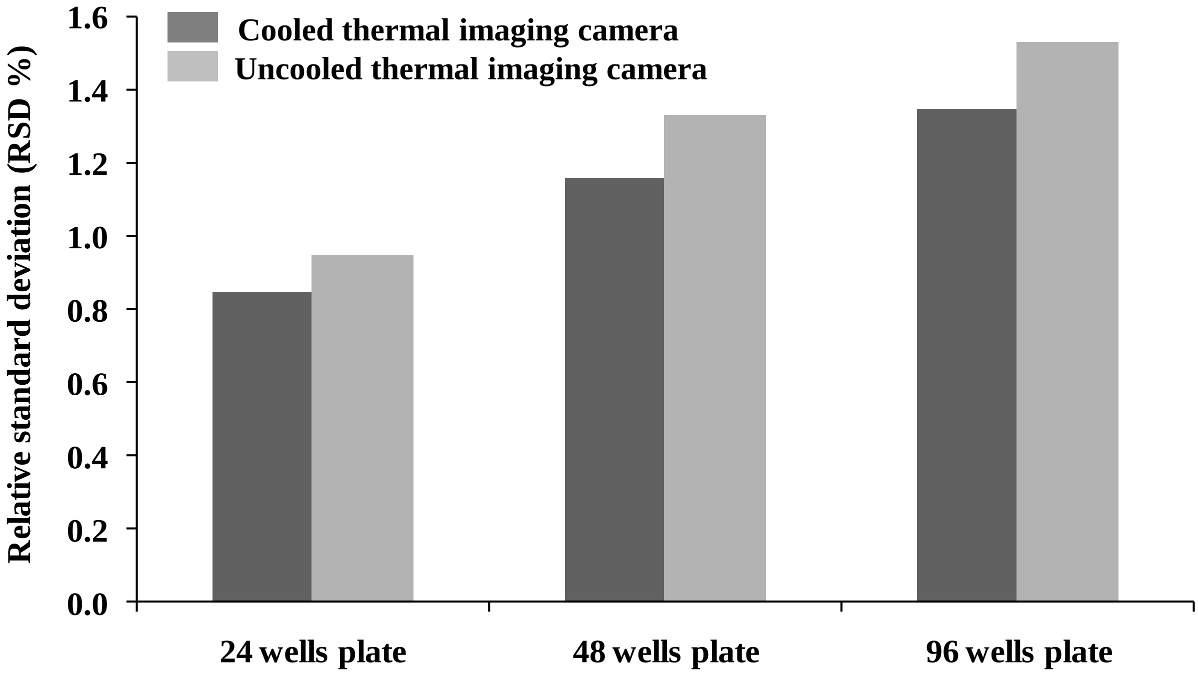

Cooled thermal imaging camera
Uncooled thermal imaging camera
Relative standard deviation (RSD %)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
24 wells plate
48 wells plate
96 wells plate

Figure 2.

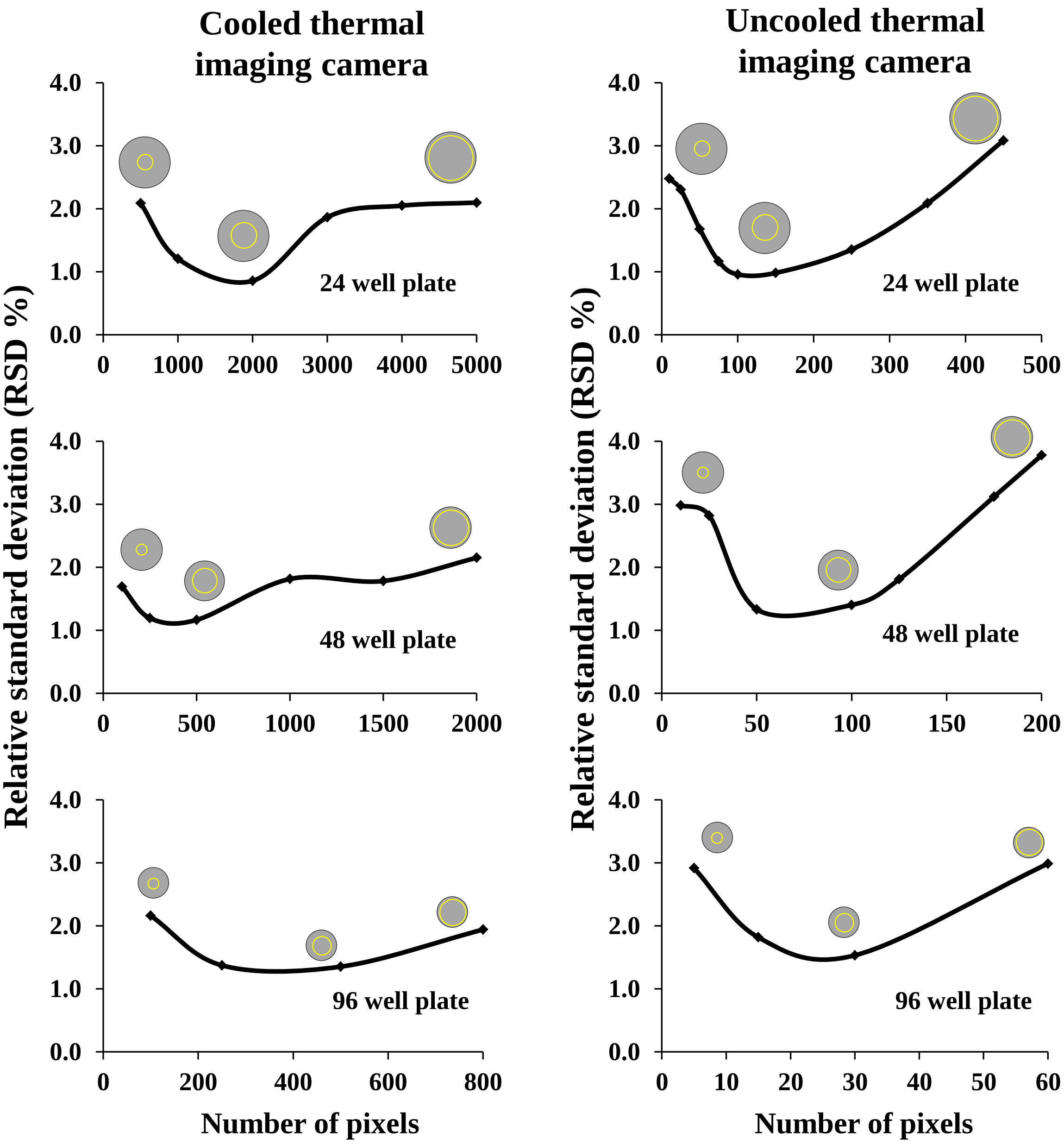

Cooled thermal imaging camera
Uncooled thermal imaging camera
Relative standard deviation (RSD %)
Relative standard deviation (RSD %)
4.0
3.0
2.0
1.0
0.0
0
1000
2000
3000
4000
5000
24 well plate
0
100
200
300
400
500
24 well plate
0
500
1000
1500
2000
48 well plate
0
50
100
150
200
48 well plate
0
200
400
600
800
96 well plate
0
10
20
30
40
50
60
96 well plate
Number of pixels
Number of pixels

Figure 3.

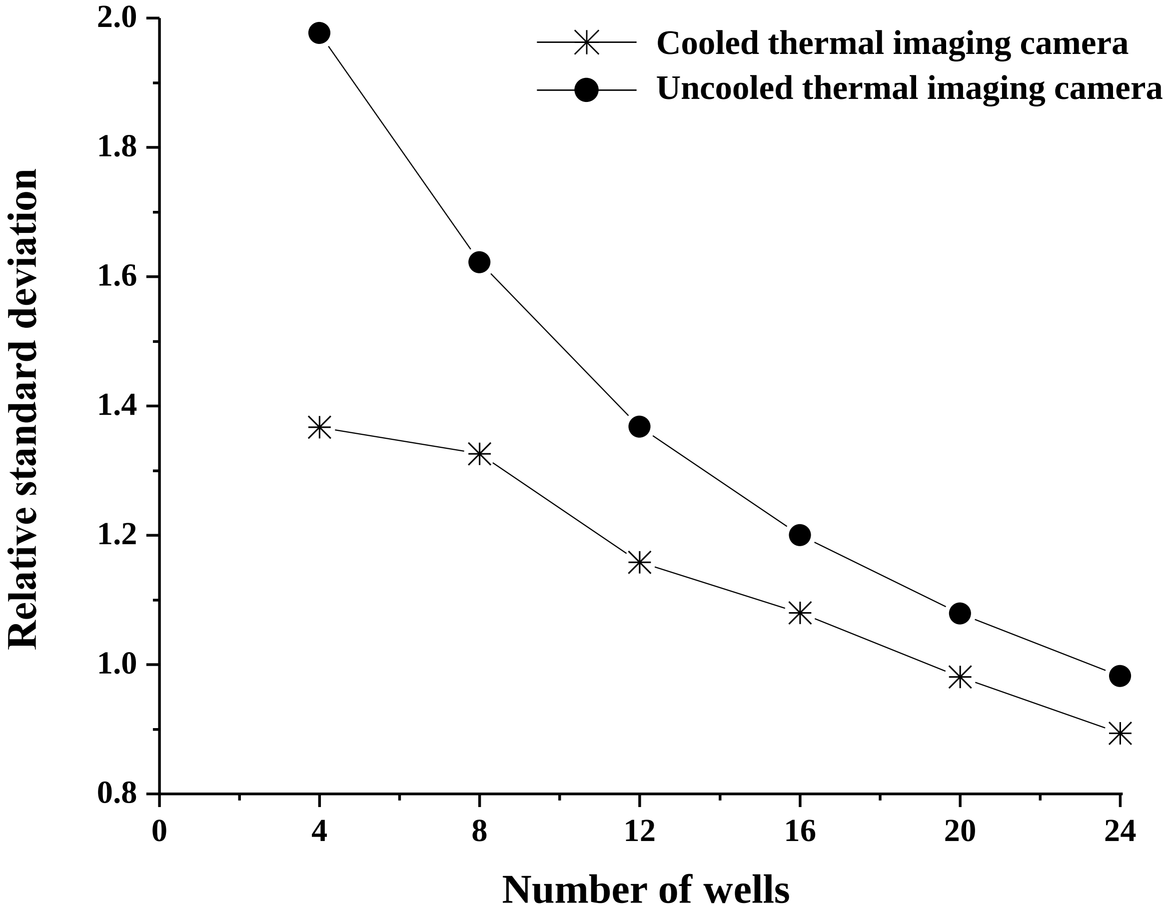
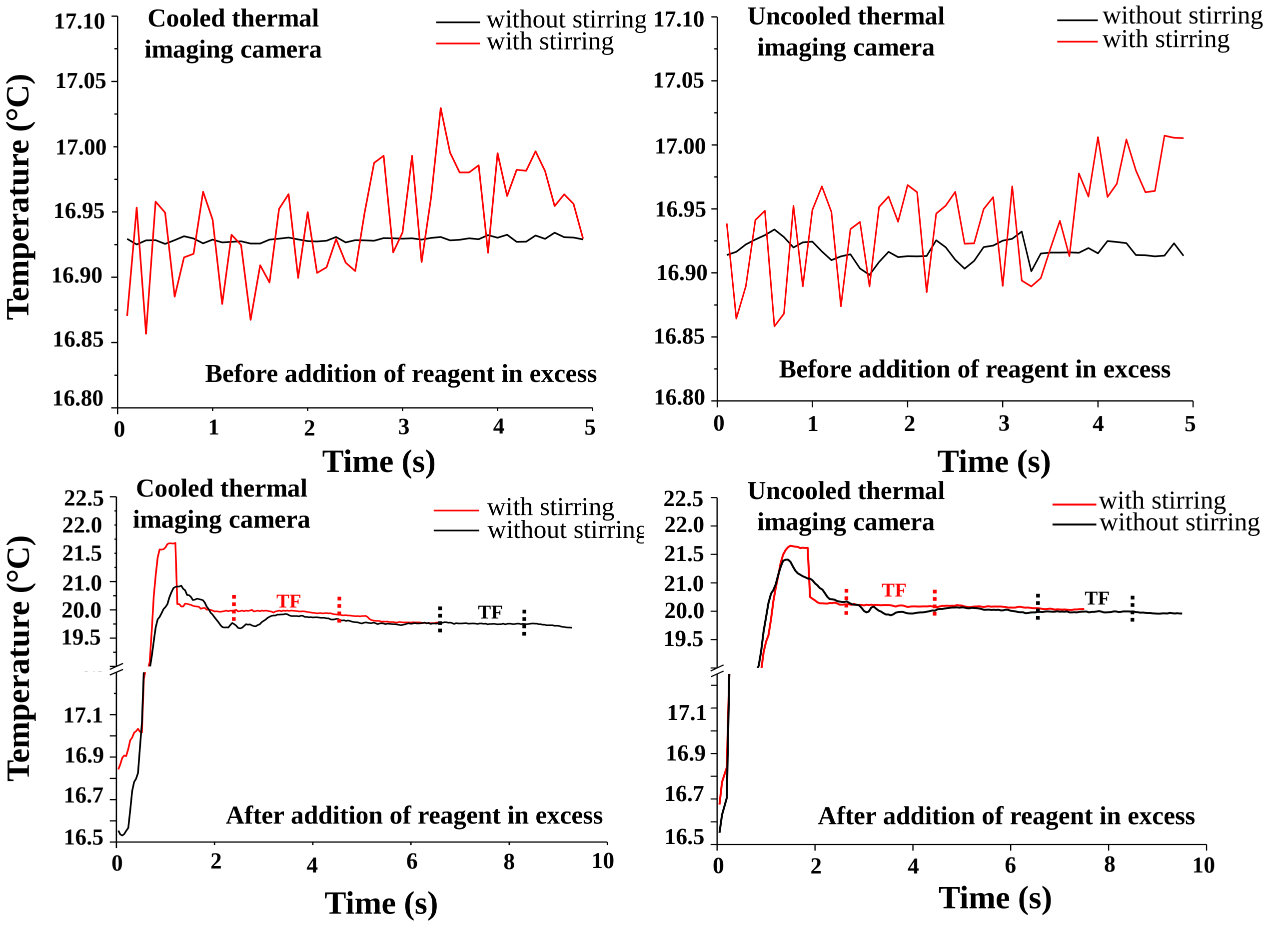

Cooled thermal imaging camera
Uncooled thermal imaging camera
Relative standard deviation
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
4
8
12
16
20
24
Number of wells

Figure 4.


Cooled thermal imaging camera
without stirring
with stirring
Temperature (°C)
17.10
17.05
17.00
16.95
16.90
16.85
16.80
Before addition of reagent in excess
0
1
2
3
4
5
Time (s)
Uncooled thermal imaging camera
without stirring
with stirring
Before addition of reagent in excess
Time (s)
Cooled thermal imaging camera
with stirring
without stirring
22.5
22.0
21.5
21.0
20.0
19.5
17.1
16.9
16.7
16.5
TF
TF
After addition of reagent in excess
0
2
4
6
8
10
Time (s)
Uncooled thermal imaging camera
with stirring
without stirring
TF
TF
After addition of reagent in excess
Time (s)

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8. DISCUSSÃO

Os resultados obtidos até o momento estão apresentados e discutidos no artigo 1, publicado no periódico *Analytical Chemistry*, intitulado “*Infrared Thermal Imaging: A Tool for Simple, Simultaneous, and High-Throughput Enthalpimetric Analysis”*; no artigo 2, publicado no periódico *Food Chemistry*, intitulado “*Rapid Microplate High-Throughput Method for Quality Evaluation of Vinegars Using Thermal Infrared Enthalpimetry*”; no manuscrito 1, submetido para o periódico *Journal of Food Composition and Analysis*, intitulado “*One-Shot Simultaneous Determination of Acidity and Salt Content of Pickled Vegetable Brine by Using Thermal Infrared Enthalpimetry*” e no manuscrito 2, intitulado “*A Critical Evaluation of Parameters for Experiments based on Thermal Infrared Enthalpimetry*”, o qual está em fase de elaboração anteriormente à etapa de submissão.

De maneira geral, pode-se dizer que a TIE apresentou características similares nos diferentes trabalhos, principalmente àquelas relacionadas ao atendimento das necessidades da química analítica verde e das análises de rotina. Nas aplicações propostas nesse trabalho, pode-se verificar que a TIE constitui uma alternativa frente aos métodos clássicos de titulação, onde reações de neutralização, precipitação, complexação e oxirredução foram exploradas com resultados concordantes com esses métodos convencionais.

Entre as características relacionadas à química verde (GAŁUSZKA et al; 2013), pode-se verificar que a TIE permite atingir ao menos nove dos doze requisitos, por ser uma técnica direta que muitas vezes não exige o preparo de amostra, necessita de mínimas quantidades de amostra, é passível a integração e automação de processos, envolve a redução do descarte de resíduos e não envolve a derivatização da amostra, podendo ser aplicada para a determinação simultânea de analitos, com redução do gasto de energia e o aumento da segurança do analista.

O aumento da frequência de análises usando a TIE foi observada em todos os trabalhos realizados até o momento e, como mostram os artigos 1 e 2, grandes diferenças são obtidas entre a TIE e os métodos convencionais. Considerando 10 s para uma análise com a TIE, é possível de efetuar 24 determinações por minuto ou 1440 por hora, o que representa uma frequência de análises muito mais elevada que o método convencional que atinge, normalmente, cerca de 60 amostras por hora. Portanto, a frequência de análises da TIE pode ser aumentada mais de 20 vezes em comparação com a titulação. Além disso, pipetas de múltiplos canais e microplacas com centenas de poços estão disponíveis e o desempenho usando a TIE pode ser ainda maior. No trabalho apresentado no artigo 2, onde foi efetuada a determinação da acidez total, fixa e volátil de vinagres, é mostrado que há um aumento na frequência de análises usando a TIE de 26 vezes em relação ao método convencional, o que torna a TIE atraente para aplicações em rotina.

A redução do consumo de energia por amostra analisada é um fator importante e que traz benefícios pelo uso da TIE, principalmente nos casos em que o preparo das amostras envolve aquecimento. Fato este, demonstrado no artigo 2, para a determinação da acidez fixa, há uma redução de 10 vezes no consumo de energia em relação ao método convencional. Essa redução do consumo energético é importante não apenas do ponto de vista ambiental, mas também econômico, uma vez que a redução de uma ordem de magnitude pode impactar significativamente os custos envolvidos. Além disso, nos trabalhos realizados com a TIE, a mesma tem se mostrado uma técnica simples, de fácil treinamento e execução pelos analistas, assim como de baixo custo para implementação e operação em laboratório. Isso também impacta os custos, uma vez que não há necessidade de treinamentos sofisticados tendo em vista a simplicidade das operações realizadas. Portanto, pode-se inferir que a TIE apresenta grande potencial para aplicação em análises de rotina, principalmente se as etapas analíticas e/ou o processamento das imagens for efetuado de forma automatizada, permitindo um grande volume de análises com um custo relativamente baixo.

Outro grande potencial da técnica é a possibilidade de fazer o preparo da amostra e a determinação no mesmo recipiente. Como mostrado no artigo 2, na determinação da acidez fixa de vinagres, a etapa de evaporação dos ácidos voláteis foi feita diretamente na microplaca para 24 amostras simultaneamente, seguida da adição do reagente em excesso para a determinação da temperatura da reação no mesmo recipiente. Essa possibilidade de integrar o preparo e a determinação em um mesmo recipiente (na microplaca) para múltiplas amostras traz a vantagem de reduzir a manipulação, simplificando a análise e evitando erros associados às medições. Esses erros podem ocorrer com facilidade em muitas situações nos métodos convencionais, como por exemplo, na determinação da acidez fixa. Isso ocorre porque, nesse caso, o resíduo da evaporação das amostras pode ficar fortemente aderido ao recipiente, prejudicando a transferência da amostra para o recipiente de vidro (erlenmeyer) onde será feita a titulação. Na TIE, esta etapa de transferência foi eliminada.

Com relação à etapa de determinação, a TIE também traz benefícios frente aos métodos titulométricos. Para muitas amostras coloridas e escuras, como por exemplo, os vinagres de vinho tinto, balsâmico e de maçã, na análise convencional a determinação deve ser potenciométrica devido à dificuldade de visualização da viragem de cor no ponto final da reação. Na TIE, a temperatura da reação não tem correlação com a cor, e sim com a quantidade de analito presente, que ao reagir com o regente em excesso irá gerar um aumento ou diminuição da temperatura. Esta característica é muito importante, pois como nas análises convencionais a determinação depende muitas vezes da acuidade visual do analista, há grande probabilidade de ocorrer erros na detecção do ponto final.

Outra característica importante para a simplificação de análises e processos é a possibilidade de analisar simultaneamente vários analitos em uma mesma amostra, o que é possível na TIE e está descrito no manuscrito 1. Neste trabalho, a TIE foi utilizada de forma diferente, introduzindo as amostras sobre os reagentes, que foram adicionados anteriormente às placas. Desta forma, é possível efetuar a determinação instantânea da acidez total e do teor de cloretos, mostrando mais uma vez a possibilidade de usar a técnica em análises de rotina e em processos produtivos, pois é possível fazer o preparo prévio dos reagentes nas microplacas e após direcioná-las ao processo para fazer a determinação simultânea e instantânea *at line* (TREVISAN e POPPI, 2006).

Como já mencionado, com o uso da TIE há redução de custos de análise desde os reagentes e amostras necessários até o consumo de energia e descarte de resíduos. Entretanto, o maior custo envolvido na TIE refere-se à aquisição da câmera. Hoje em dia, há no mercado uma grande oferta de equipamentos deste tipo e por um preço acessível, principalmente as câmeras que possuem detectores que operam a temperatura ambiente, como os microbolômetros. Como mostrado no manuscrito 2, um desempenho satisfatório para a TIE pode ser obtido mesmo com o uso destes equipamentos. Desta forma, existe uma grande possibilidade de difusão da TIE devido ao acesso cada vez maior de laboratórios a estes equipamentos.

Entre as limitações da TIE, pode-se destacar a necessidade da utilização de reações seletivas, pois reações paralelas com o reagente em excesso podem ocorrer além daquelas esperadas com o analito, o que pode gerar um aumento ou redução na temperatura que irá causar interferência na determinação. Por este motivo, é importante saber quais as substâncias presentes nas amostras que podem reagir com o reagente em excesso, sendo necessário que apenas a reação entre o composto de interesse e o reagente em excesso ocorra ou que as reações em paralelo não afetem a temperatura da solução. No manuscrito 1, foram determinados cloretos e de acidez total de amostras de picles, onde foi efetuado um estudo de interferências de outros compostos presentes na amostra. Nenhuma interferência foi observada nesse caso, mostrando a especificidade do método utilizado.

Apesar das limitações supracitadas com relação à seletividade das reações na TIE, cabe destacar que as reações estudadas são as mesmas empregadas nos métodos titulométricos, e resultados concordantes foram obtidos. Desta forma, pode-se inferir que é esperada um desempenho similar da TIE frente a esses métodos clássicos, de maneira que a seletividade afeta os dois métodos de maneira semelhante. Outra possibilidade seria o uso de reações específicas de *per si*, como por exemplo, as reações enzima-substrato e antígeno-anticorpo.

Contudo, a TIE pode apresentar limitações para as reações enzima-substrato e antígeno-anticorpo, pois muitas vezes essas reações são lentas. Nesse caso, o calor gerado pela reação pode ser dissipado para a vizinhança (p. ex., a microplaca ou o ar ambiente), uma vez que o sistema não se encontra isolado como nos calorímetros usados na entalpimetria clássica. Assim, o calor liberado pelas reações poderá impedir o aumento significativo da temperatura das soluções, dificultando as medições por TIE.

É importante também ressaltar que como a TIE é uma técnica nova e que há muitos desenvolvimentos a serem feitos e muitas possibilidades para a técnica. Como comentando, entre os principais tipos de análises entalpimétricas estão às titulações termométricas e a entalpimetria em fluxo, entretanto as mesmas geralmente são feitas usando microcalorímetros que são recipientes de custo elevado e de pouca simplicidade de uso e para a determinação da temperatura gerada na reação. Além disso, esses sistemas de análise entalpimétrica usam termistores que são equipamentos invasivos e que determinam a temperatura pontual da amostra. Desta forma, novas possibilidades de desenvolvimento relacionadas com essas duas técnicas de análise entalpimétrica são esperadas, ampliando as aplicações da TIE.

Outra possibilidade de desenvolvimento é a utilização de reatores diferentes daqueles empregados nesse trabalho. Por exemplo, recipientes isolados termicamente poderiam ser desenvolvidos para reduzir a dissipação do calor gerado nas reações de forma a permitir o monitoramento de reações mais lentas. Contudo, é importante, nesse caso, manter as características de baixo custo e praticidade encontradas nas aplicações propostas. Ademais, outros materiais além do poliestireno, comumente usado para a fabricação das microplacas podem ser explorados de forma a assegurar maior resistência química e térmica. No caso da TIE, as microplacas não precisam ser transparentes, pois as medidas não são efetuadas através da passagem de luz pelo recipiente. Cabe ressaltar ainda, a possibilidade de uso de outros dispositivos para reação, como aqueles que empregam papel. Nesse caso, poderm ser utilizados volumes menores de amostra sem alterar significativamente a área superficial monitorada pela câmera.

9. CONCLUSÃO

A combinação da termografia no infravermelho com a análise entalpimétrica mostrou-se promissora para determinação de diversos analitos e também por realizar a análise simultânea de uma forma simples, rápida e com resultados concordantes com técnicas convencionais. Diferentes reações foram exploradas, com resultados satisfatórios para neutralização, complexação, precipitação e oxirredução. A otimização e a validação da TIE permitiram obter as condições ótimas de análise, obtendo melhor sinal analítico. Cabe destacar que a TIE pode ser utilizada para monitoramento de outros fenômenos químicos ou físicos envolvendo calor, tais como adsorção, dissolução e fase transição, demonstrando o potencial para diversas aplicações.

Câmeras no infravermelho que apresentam melhor sensibilidade térmica, maiores taxas de aquisição de imagens (p. ex., câmeras com detector refrigerado) geraram melhores resultados. Contudo, de acordo com os estudos apresentados, uma câmera com detector do tipo microbolômetro (não refrigerado) demonstrou resultados satisfatórios e promissores para condução da TIE, uma vez que o custo envolvido na aquisição desse equipamento é relativamente baixo. A simplicidade de operação e tratamento dos dados permite a aplicação da TIE em laboratórios de rotina, uma vez que as análises requerem operações simples e pequeno grau de treinamento para execução, o que a torna atraente para o processamento de várias amostras.

A TIE se mostrou em conformidade com vários dos conceitos da química analítica verde, como em relação à simplicidade, rapidez, redução de reagentes e de consumo de energia, assim como na redução do volume de resíduos gerados. Tendo em vista que esses foram os primeiros trabalhos propostos com a TIE, é esperado o atendimento dos demais requisitos em função da possibilidade de novos desenvolvimentos. Assim, parâmetros como a redução de reagentes tóxicos e utilização de reagentes obtidos de fontes renováveis, assim como adaptação a determinações *in situ* podem ser esperadas em um futuro próximo. Isto leva a TIE a ser considerada uma nova técnica “verde” disponível no ferramental analítico.

De acordo com os resultados obtidos, pode-se observar que uma das limitações da TIE está relacionada com a seletividade das reações, fator muito importante para a entalpimetria.

Além disso, nos primeiros estudos, todos os resultados foram obtidos pela operação manual de pipetagem e de tratamento de dados. Portanto, para os próximos estudos, a automação destas etapas pode ser efetuada, gerando um aumento no desempenho da TIE, reduzindo o desvio padrão das medições entre as determinações e aumentando a frequência de análises, potencializando ainda mais seu uso em rotina.

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